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FROM THE DEPARTMENT OF DERMATOLOGY (HEAD: PROFESSOR SVEN HELLERSTRÖM)
AND ALLERGY RESEARCH LABORATORY (HEAD: PROFESSOR ÅKE NILZÉN)
KAROLINSKA SJUKHUSET STOCKHOLM

EPIDERMAL TREATMENT OF GUINEA PIGS WITH POTASSIUM BICHROMATE

*The influence of a nonionic detergent, a lauryl alcohol-ethylene
oxide condensate, on the skin reactions caused by
potassium bichromate treatment*

BY

KJELL WIKSTRÖM

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STOCKHOLM 1962

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KJELL WIKSTRÖM

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CHAPTER I

Review of the literature and aims of investigation

The ability of simple chemical substances to give rise to a sensitization through cutaneous treatments is usually investigated in the guinea pig. The experimental procedure used has varied. In general the animals have been given intracutaneous injections of the substance in question or this has been applied epidermally.

A number of simple chemical compounds have been tested in this way and the sensitization of guinea pigs has succeeded well with, *inter alia*, neoarsphenamine (Frei, 1928; Sulzberger, 1929), paraphenylenediamine (Mayer, 1928; 1931; Dienes, 1933) and with a number of aromatic compounds with substituted chloro and nitro groups (Landsteiner & Jacobs, 1935, 1936; Landsteiner & Chase, 1937). In experiments to sensitize the guinea pig with heavy-metal salts the results have varied. As these investigations with nickel and chromium are very intimately correlated, they are both described in detail below.

Earlier experiments on the sensitization of the guinea pig to nickel

These investigations are summarized in Table 1. Walthard, (1926) seems to be the first researcher to try to sensitize the guinea pig to nickel. The animals were treated with 1 % nickel sulphate solution epidermally daily for 2—3 weeks. As a result of the treatment all 31 animals had local dermatitis and these began to be noticeable after 2—3 weeks of treatment. At testing on the contralateral untreated side the skin changes might appear after only 3—4 days' treatment. Thus in the same animal the number of days of treatment needed to provoke a dermatitis had decreased. According to Walthard this suggested that a sensitization to nickel had taken place. He also remarks that subcutaneous injections of 1 % nickel solution can produce a flare up within the skin areas treated earlier. This he claims as further evidence of the occurrence of a nickel allergy.

Results in agreement with this have been obtained by later authors. Graul & Kalkoff (1948) treated guinea pigs with nickel sulphate daily for 4—6 days. The concentration was increased daily as follows: 1 % — 2.5 % — 5 % — 10 %. In this way the authors arrived at a limit concentration for primary toxic irritation. With a 1 % nickel solution, the concentration of which was about one-tenth of this threshold value for primary irritation, epidermal testing was carried out 12 days after the beginning of the experiment. Within 24 hours dermatitis appeared in the majority of the experimental animals. An allergy was considered to exist.

Nilzén & Wikström (1955) treated guinea pigs daily epidermally with 4 % nickel sulphate solution with or without addition of sodium lauryl sulphate 1 % (SDS). In one of the animals which had been painted with nickel + SDS a dermatitis appeared after 7 days. Epidermal testing with 4 % nickel on the 12th day of the experiment produced a dermatitis within 24 hours in this animal. Others had no macroscopic changes.

Vinson (1959) sensitized guinea pigs with nickel sulphate 0.5 % in combination with 1 % SDS or with 1 % sodium dodecyl benzene sulphonate (= DDBS). As a result of

epidermal treatment daily for 10 days local dermatitis appeared. Testing was carried out on the 20th—24th day after the beginning of the experiment, the animals being treated epidermally with the same solution as previously. After 2 challenge applications pronounced dermatitis appeared. On the other hand, the author did not succeed in sensitizing the guinea pigs with nickel sulphate alone in concentrations 0.5 % and 5 %. No potentiating effect of 0.5 % nickel sulphate was obtained if the solution also contained some detergents such as the anionic sodium acyl isethionate, sodium acyl n-methyl taurate, commercial toilet soap, the nonionic detergents lauryl alcohol-ethylene oxide condensate (Atlas Brij 35)®, tridecyl alcohol-ethylene oxide condensate (Monsanto's Sterox AJ—100)® or the cationic distearyl dimethyl ammonium chloride (Armour's Arquard 2 HT)®.

The experiments now described, where sensitization to nickel succeeded, contrast with the experience of other authors.

Stewart & Cormia (1934) treated guinea pigs both by daily epidermal applications of nickel chloride in concentrations of 2 — 10 — 20 % for 7—28 days and through subcutaneous, intradermal and intraperitoneal injections of 2 % nickel chloride solution. The epidermal treatment led to local dermatitis. At the subsequent testing epidermally with 10—20 % nickel chloride solution nothing appeared which could support the hypothesis of an acquired sensitization to nickel. The authors came to the same conclusion after intracutaneous testing. In the same work it is mentioned that *Milford & Coca* did not succeed in sensitizing guinea pigs to nickel. Those experiments, however, are not published.

Hunziker (1960) tried in various ways to sensitize guinea pigs to nickel. Some of the animals were treated epidermally with 1—30 % nickel sulphate solution with or without an addition of Triton X 100. Even in those cases where local dermatitis arose as a result of this treatment no nickel allergy could be shown. Other guinea pigs were treated with injections of nickel, *inter alia* in combination with Freund's adjuvant. Here too, epidermal and intracutaneous testing gave negative results.

Earlier experiments on the sensitization of guinea pigs to chromium

Hitherto chromium salts have been very little used in attempts to sensitize guinea pigs. *Nilzén & Wikström* (1955) were probably the first researchers to produce dermatitis in these animals through epicutaneous applications of potassium bichromate (Table 2). After daily treatment for 8 days with a mixture of 0.5 % potassium bichromate and 1 % SDS all 5 animals had undergone skin changes. Epidermal testing with only potassium bichromate 0.5 % 12 days after the beginning of the experiment gave local dermatitis in all 5 guinea pigs within twentyfour hours. A sensitivity to chromium was considered to have occurred. Treatment of guinea pigs with only 0.5 % potassium bichromate from the first painting did not lead to any obvious macroscopic skin changes locally. In these the testing produced no positive result.

Similar results were later obtained by *Levin, Brunner & Rattner* (1959). They treated guinea pigs epidermally 5 times in all on alternate days with potassium bichromate 1 %. Testing 20 days after the beginning of the experiment with the same chromium con-

centration did not cause any dermatites. In another group of animals the skin was defatted by vigorous swabbing with petroleum ether before each application of the potassium bichromate solution. In other respects the experiment did not differ from the preceding one. At the subsequent testing, dermatites were found in 6 of the 12 guinea pigs with a chromium solution which was diluted down to 0.05 %. The number of test applications is not stated.

Other authors have to some extent tried to reproduce the chromium investigations mentioned, but have not been able to demonstrate a sensitivity to chromium.

Thus, *Samitz & Pomerantz* (1958) were unable to sensitize two guinea pigs with 0.5 % potassium bichromate solution or 4 other animals with a mixture of the same chromium solution and 1 % SDS. The animals were treated daily for 8 days and testing was carried out rather more than 3 weeks later.

Mayer & Jaconia (1958) treated guinea pigs epicutaneously on alternate days on 3 occasions in all, with 0.5 % solutions of potassium bichromate, chromium chloride and the trivalent, complex chromium salt $\text{Cr}_2(\text{OH})_2(\text{SO}_4)_2$. The first two of the solutions mentioned also contained 1 % SDS. Whether this treatment caused local dermatites is not clear. Three weeks after the beginning of the experiment the animals were again treated in the same way as earlier. At this testing one of the guinea pigs treated reacted in such a way that a sensitization to chromium was considered to exist. This animal had been treated with the complex, tanning chromium salt. The authors also attempted to sensitize guinea pigs by intradermal injections of the respective chromium salts in the concentration 0.025 %. The animals were given three injections in all on alternate days. Intradermal injection with the various solutions 3 weeks after the commencements of sensitization released positive test reactions in the majority of the guinea pigs.

Hunziker (1960) tried to sensitize guinea pigs in a similar way, but was unable to show an epidermal sensitivity to chromium in any of the 9 guinea pigs which had been treated epicutaneously daily for 8 days with a mixture of potassium bichromate 0.5 % and Triton X 100 1 % or SDS 1 %. The testing was carried out on the 9th day of the experiment. On the other hand, it proved that injections in the neck with potassium bichromate together with Freund's adjuvant resulted in a sensitivity to chromium. It was possible to show this 3 weeks later by epidermal application of 0.5 % potassium bichromate solution as well as by intradermal testing in the concentration 0.025 %.

Distinguishing between a primary toxic or allergic dermatitis

Most of the substances which can give rise to an epidermal sensitization may in sufficiently strong concentrations also cause primary irritation to the skin. It may therefore be difficult to establish whether a dermatitis which has arisen as a result of repeated epidermal contacts with a substance reflects a sensitization or a primary toxic effect.

An epidermal treatment in order to bring about a hypersensitivity to a substance can proceed without skin irritations appearing during the course of sensitization itself (*Landsteiner & Jacobs*, 1935). It is, however, more common for local dermatites to appear during the progress of the sensitization. The number of applications needed in order to obtain

such skin changes varies, a number of factors influencing the course. Examples of these factors are the concentration of the substance in question, the derivation of the animal, whether treatments are carried out daily or on alternate days etc.

In a pure irritation of the skin a dermatitis may develop with a course similar to that which has just been described as occurring on sensitization. Repeated contact with the substance in question give a skin change of primary toxic origin. The defence of the skin has more and more been diminished locally (*Shelanski & Shelanski, 1953*). Not infrequently, however, the skin damage appears quite soon after the contact of the skin with the substance in question. Researchers speak of a cumulative or an immediate reaction respectively.

In experiments with epidermal sensitization the term "latency time" is found. By this is meant the time from the beginning of the treatment to the point where a local dermatitis appears. When the animals, after having undergone treatment for sensitization, are again exposed to epidermal applications of the substance in question, this latency time may be found to be shorted. If this is the case, it is considered that a hypersensitivity to the substance exists. This was the case when *Walthard* (1926) treated guinea pigs with nickel. He also found that an animal which had earlier been treated with nickel, in which the dermatitis had been healed, could have a flare up of this on injection of nickel solution in skin areas previously untreated. *Walthard* also points out that the animals reacted differently to the same primary treatment. If instead the animals during this treatment had had dermatitis of the same strength throughout, this would, on the other hand, have rather tended to show that the skin changes had been caused by a pure irritation. *Graul & Kalkoff* (1948) also mention the importance of a shortened latency time at testing as demonstrating an epidermal hypersensitivity. This latency time is shorter when a very pronounced sensitivity exists than when the sensitivity is less pronounced (*Nilzén, 1952*). *Nilzén* also recommends that the test solution should be weaker than that used in order to obtain a sensitization. This agrees with the experience of other authors, and according to *Rostenberg Jr & Haeberlin* (1950) a sensitization exists when a substance in concentrations which previously were not irritant produces reactions on skin previously untreated.

Several authors have compared the results of epicutaneous tests with those obtained through intracutaneous testing. These two methods of testing seem to give results which are in agreement with each other. In experiments with sensitization to nickel the tests turn out negatively, i.e. no hypersensitivity to nickel can be shown (*Stewart & Cormia, 1934; Hunziker, 1960*). As regards similar experiments with chromium, a sensitivity to this can be shown epidermally as well as intracutaneously (*Hunziker, 1960*).

Certain factors which may influence a sensitization of guinea pigs to simple chemical substances

It is well known from experimental sensitizations of guinea pigs that animals react differently to the same treatment. This individual mode of reaction may be so pronounced that it is difficult to reproduce the experiments. Thus, *Sulzberger* (1930) succeeded in

sensitizing guinea pigs to nearly 100 % to neoarsphenamine at Breslau; in similar experiments in New York none of the animals showed hypersensitivity. In Zürich *Sulzberger* obtained results which lay between these extremes.

Factors affecting a sensitization may be endogenous and exogenous. As far as endogenous factors are concerned, heredity is of particular importance. There is often a definite difference between guinea pigs of different strains. *Chase* (1941) has shown the possibility of producing by breeding, animals with very pronounced reactivity as well as animals with practically no disposition to be sensitized. Such extreme differences may exist even within the same strain of animals. *Chase* also mentions that females generally seem to react better than males.

Some authors have observed that diet may affect the sensitization. Lack of vitamin C can reduce allergic reactions (*Kile & Pepple*, 1938). Vegetables inhibit a sensitization and a dry diet promotes sensitization (*Mayer & Sulzberger*, 1931). The same authors succeeded better with winter animals than with summer animals. There is, however, a divergence of use as regards the importance of the seasons for epidermal sensitization, and some authors have succeeded best with summer animals (*Frey*, 1951).

Whether an epidermal sensitization will succeed or not depends largely on the experimental procedure used. *Schnitzer* (quoted *Frey*, 1951) showed that the quantity of allergen in proportion to the skin area treated was of essential importance. A concentrated allergen solution which was applied to a point-shaped skin surface could produce better results than a diluted solution applied to a larger area. *Graul & Kalkoff* (1948) defatted the skins of guinea pigs with ether before they treated the animals with nickel solutions. The same procedure was used by *Levin et al.* (1959). These researchers considered that in this way they could facilitate a penetration of the water-soluble allergen into the skin. In these investigations, however, there is nothing to show that pre-treatment with ether really promoted a penetration. *Levin et al.* also thought that a traumatization, possibly a treatment of the skin with acids and alkalis, could facilitate the penetration of a substance into the skin. In this way a sensitization could be favourably influenced. These, however, are only theories. On the other hand *Graul et al.* (1950) have shown that a scarification of the skin facilitates the epidermal sensitization with a sulphanilamide.

Skin irritations similar to those just described, i.e. exposure to alkalis, acids, detergents and fat-dissolving agents, as well as friction, may easily produce an acanthosis. A provoked acanthosis may be supposed to facilitate sensitization (*Baer et al.* 1956). The result of such a sensitization with a mechanical or chemical pre-treatment of the skin, after which the allergen is applied, is dependent on the time interval between the two steps. The best results are obtained if the sensitizer is applied immediately or within a few hours after the irritant pre-treatment (*Rockwell*, 1955).

The solvent of a substance can inhibit or promote its penetration into the skin (*Eller & Wolff*, 1939; *Bürgi*, 1937; *Zondek*, 1938; *Haas*, 1956; *McKee et al.*, 1945). The addition of a surface-active agent may also facilitate penetration (*Duemling*, 1941; *Lohaus*, 1955). The possibility of promoting sensitization in this way has been dealt with by, among other authors, *Sulzberger* (1939) and *Rabeau & Malangeau* (1947). Experimental studies of animals have provided support for this theory. *Nilzén & Wikström* (1955) showed that the presence of SDS facilitated the development of dermatitis at epidermal

treatment with nickel sulphate — and to a still greater degree with potassium bichromate solutions. The animals which had such skin changes were considered to be sensitized to the respective metal salt. *Vinson* (1959) has since, in part, taken these experiments, further. He was able to show a difference between the effect of different detergents when it was a matter of influencing the development of a nickel dermatitis and a nickel sensitivity in the guinea pig. The best results were obtained with certain anion active substances such as sodium lauryl sulphate and sodium dodecyl benzene sulphonate. The nonionic and cationic substances tested have so far been without effect.

The importance of surface-active substances for the occurrence of nickel and chromium allergies in industry has been discussed. *Levin et al.* (1959) consider that the fat-dissolving capacity with accompanying defatting is a contributory factor. This defatting reduces the alkali resistance of the skin (*Dünner*, 1950). This is the case in, for example, the galvanizing industry, and it appears that this may facilitate a sensitization to *inter alia* chromium and nickel (*Wendlberger & Weitgasser*, 1956). These surface-active agents also involve an irritation of the skin and may cause an acanthosis. One theory is that such a skin change constitutes one of the non-specific factors which can facilitate a sensitization (*Baer et al.*, 1956). An anion-active detergent such as SDS has, in addition to its effect in reducing surface tension, also the ability to denature protein (*Putnam*, 1948). By this means that SDS also influences the keratin. Whether it is one of these two properties of SDS which has made it possible to promote a sensitization to nickel or chromium is a question that has not yet been cleared up. Both possibilities have been discussed by *Nilzén & Wikström* (1955). Nickel sulphate in the presence of the anion-active substances SDS and DDBS has proved to be able to penetrate deeply into the skin and has then been shown within the pilosebaceous apparatus in the dermis and in the basal parts of the epidermis (*Vinson*, 1959). This distribution agrees well with the percutaneous absorption that *McKee et al.* (1945) obtained from ferric ammonium citrate, sulphanilamide, acid blue and iodobismutol. These authors also found that dodecyl benzene sulphonate facilitated such a penetration. Other surface-active agents tested by *Vinson*, whether anionic, nonionic or cationic, did not give any increased nickel penetration. Nor had these agents any potentiating effect on the occurrence of nickel dermatites. These differences support the view that something other than the ordinary surface-active effect is decisive for the penetration of a substance into the skin. *Vinson* mentions in this connection factors which have already been dealt with earlier, namely the importance of an acanthosis and/or a denaturation of the keratin.

Aim and scope of present investigation

The present work sets out to investigate whether the capacity of potassium bichromate to produce dermatites at epidermally applications to the skin of the guinea pigs can be increased by an addition of a surface-active, no-protein-denaturing substance. More over the question will be investigated whether epidermal treatment with potassium bichromate can cause specific epidermal hypersensitivity.

CHAPTER II

Author's investigation

Treatment of Guinea Pigs with Potassium Bichromate, Potassium Bichromate and La8Et as well as with La8Et.

Material and methods

Experimental animals. The guinea pigs used were supplied from three different strains. The distribution is shown in Table 17. White female guinea pigs were used in all cases. The weight was between 200 and 400 grams. The feeding stuff was compressed in the form of cubes (supplier: A. Etholén) with an addition of carrots ad lib. (*Hård & Häggquist*, 1957). Control animals and other animals were kept separately in groups. In the large cages the maximum number of guinea pigs was 15. The experiments were carried out during the winter months.

Data on the solutions used, see *Blohm & Wikström*, 1962.* "Potassium bichromate, Merck's Reagent No. 4864, pro analysi were used in order to produce the experimental dermatites.

The surface-active substance is an alkyl polyethylene glycol ether, here called La8Et (the code is derived from the fact that the substance is built up of lauryl alcohol to which has been added 8 moles of ethyleneoxide, calculated amounts. A theoretical molecular weight of 538 is calculated from this. The substance is non-ionic in nature and has no known protein-denaturing effect.

The potassium bichromate content and the surface-active effect of the solutions were checked during the course of experiment by taking samples from the storage receptacles used in the animal experiments. The content of bichromate, which was determined by direct spectrophotometry (after the customary diphenylcarbazide method had proved to give misleading results in the presence of the surface-active agent), varied within not more than 5 % of the stated value during an observation period of 3 weeks from the preparation. In experiments during a longer period of time, changes in the solutions in the form of precipitation and reduction of the bichromic content were observed.

Addition of the surface-active agent reduced the surface tension (relative to water) for bichromate-water-solutions to about half the value. The total surface-tension-reducing effect could be established down to dilutions of about 0.14 mM La8Et. Detergent alone in the water showed the same surface-active effect qualitatively as well as quantitatively. The surface-tension-reducing effect of the bichromate-water solution itself was, within the margin of error of the experiment, not distinguishable from that of water (e.g. for a 17.4 mM solution 97 % of that of the water).

Glass-distilled water was used as solvent and the solutions were prepared by mixing initial solutions with double strength. In connection with the continuing control of

* The following account is given by Blohm.

bichromate content and surface activity, the presence of animal hairs from the experiments was noted in some cases. Such a contamination did not cause with methods available any observable change in the chemical and physical properties of the solution."

The concentration for chromium is 0.0174 M and for La8Et 0.0087 M. These concentrations were found suitable after a preliminary experiment which is reported on page 12.

Experimental procedure. Guinea pigs in groups of 5—15 were used at each occasion during the experiment. The animals in one group were treated with potassium bichromate alone, those in another group with potassium bichromate together with the surface-active agent La8Et. Finally, animals in a third group were treated with the surface-active substance alone.

The fur along one side was removed with an electric hair-clipper after which 0.2 ml of the respective solutions were dropped on the skin. With a glass rod the fluid was distributed over a treatment area of about 2×4 cm for 2 minutes. The skin was treated in this way daily for ten days, including Sundays. During the course of the experiment the fur was clipped every second or third day. The skin was inspected before each treatment and 24 hours after the last one.

The reactions were graded as follows:

- (+) = diffuse, unevenly distributed reddening
- + = fully noticeable reddening over the greater part of the treated area
- ++ = reddening with infiltration.

A number of biopsies were made for histological examination, as well as from skin, which had been treated in the same way with distilled water.

Preliminary experiments to determine suitable concentrations of potassium bichromate and La8Et

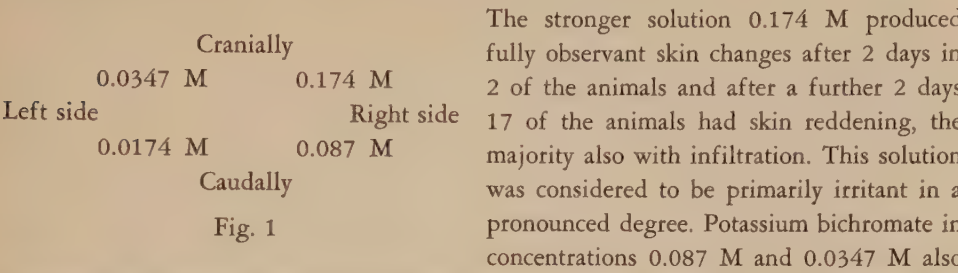
Different concentrations of potassium bichromate and La8Et were painted on guinea-pig skin in order to try out suitable concentrations for the future experiments.

La8Et. This surface-active agent was tested in the concentrations 0.0347 M, 0.347 M and 0.694 M. The experimental procedure has already been described on p. 12. The respective solution were applied epidermally on guinea pigs in groups of 5 and the results are to be seen in Table 3.

A solution which is 0.0347 M gave no macroscopically observable reaction after 2 days' treatment, but on the following day there could be observed in one of the five animals a weak, unevenly distributed reddening. After 6 days there occurred in the same guinea pig a definite reddening over the treated area and at the same time a further animal reacted with a weak reddening. The 0.347 M solution, which was 10 times stronger, gave fully observable skin changes after only 2 days' treatment in all 5 guinea pigs, and such a solution may therefore be regarded as a primary irritant. None of the tested solutions was considered suitable for the further experiments. A considerably lower concentration, i.e. 0.0087 M, was used thereafter. As already mentioned (p. 11), such a concentration produces a maximum surface-tension-reducing effect.

Potassium bichromate. On 18 guinea pigs concentrations of 0.174 M, 0.087 M, 0.0347 M and 0.0174 M were tested. The procedure differs to some extent from that dealt with earlier (p. 12), and will therefore be described here.

On each side of the experimental animals the fur was clipped within 2 distinct areas. In this way there was obtained in each guinea pig 4 areas of treatment. These were painted as shown in Fig. 1 with the respective potassium bichromate solution in the same way as stated earlier (p. 12). The results are shown in Table 4.



The stronger solution 0.174 M produced fully observant skin changes after 2 days in 2 of the animals and after a further 2 days 17 of the animals had skin reddening, the majority also with infiltration. This solution was considered to be primarily irritant in a pronounced degree. Potassium bichromate in concentrations 0.087 M and 0.0347 M also produced skin reactions after 4 days' treatment, but these dermatites were less pronounced than the preceding ones. As regards the weakest chromium solution, 0.0174 M, no macroscopic changes could be observed during the 5 days of the experiment. This concentration was therefore considered to be suitable for the future investigations.

Results of epidermal treatment of guinea pigs with solutions containing potassium bichromate 0.0174 M and La8Et 0.0087 M

Table 5 gives the results for those animals which were treated with potassium bichromate only and those treated with a mixture of potassium bichromate and La8Et. Each experiment is reported in chronological order, and the skin reactivity in the animals can be followed during the 6th to 11th days of the experiments, i.e. after 5—10 days' treatment. Table 6 contains in the same way guinea pigs which were treated with potassium bichromate and La8Et and animals which were treated with La8Et alone.

Table 17 shows how many animals reported in Table 5 came from each of the three suppliers. The majority of the guinea pigs, i.e. 167, were supplied from the same farm, and these were distributed among 7 of the 9 series of experiments. At the other two experimental occasions 20 and 19 guinea pigs respectively came from each of the other two suppliers.

Some animals, in all 5, without other signs of disease became thin and increasingly weak. One animal died, the others were destroyed.

Tables 7—15 show a number of animals with or without reactions after 5—10 days' treatment with chromium and with chromium + surface-active agents respectively. In Table 16 all these experiments are assembled.

From these tables it appears that the results are not identical from one experiment to another. In some series we find that the chromium solution as such did not in any case give rise to skin changes which can be described as definitely positive (Tables 8, 9, 10, 13 and 15). On the other hand Tables 7, 11, 12 and 14 show that potassium bichromate

in the concentration here used, 0.0174 M, can provoke dermatitis. The skin changes which then arise appear at the earliest after 7 days' treatment, i.e. on the 8th day of the experiment (Table 12). In all 103 guinea pigs were treated with chromium solution alone and of these 2 animals died (Table 16). After 10 days' treatment 11 of the remaining 101 animals had definite positive reactions. The majority (8) of these skin changes consisted of reddening with infiltration, and in the 3 remaining animals fully observable reddening was noted. The number of animals with diffused, unevenly distributed reddening mounted to 9. Eighty-one guinea pigs were wholly negative, that is to say were without any macroscopically observable signs of skin damage.

Treatment with the mixture of potassium bichromate and the surface-active agent La8Et led in all the series of experiments to dermatitis, although the frequency of these varied. These skin changes could appear after only 5 days' treatment and then consist of reddening (Tables 8, 11, 12 and 15). After 10 days, 3 of the total of 103 animals died (Table 16). In all, 50 guinea pigs had had dermatitis. In the majority of these (37), reddening with infiltration could be observed. The number of animals without any visible sign of skin damage was 34, and diffuse, unevenly distributed reddening was observed on 16 animals.

As will be seen from Table 6 and Tables 19—22, 31 guinea pigs were treated with the solution containing La8Et alone. None of these animals had any macroscopically observable skin changes after 10 days' treatment.

Histological investigations

In the foregoing it has been mentioned that the epidermal treatment of guinea-pig skin with 0.0087 M La 8 Et did not cause macroscopically observable skin changes. Microscopically, however a certain change in the status of the skin occurred after 7—8 days' treatment. This took a form of an irregular, moderate acanthosis with a thickening of the prickle cell layer. No spongiosis could be shown and the basal cell layer was intact (Plate 1). Stronger concentrations of La 8 Et (0.0347 M), on the other hand, could give rise to a macroscopically observable reddening. Here the histologically observable changes were dominated by a marked acanthosis with thickening of the prickle cell layer. No noteworthy spongiosis, however, could be shown. In the upper part of the cutis the capillaries were dilated and here and there was a sparse cell infiltration which mainly consisted of round cells and isolated leukocytes. The basal cell layer was intact (Plate 2).

Treatment with potassium bichromate following which no macroscopically changes are observed nevertheless causes histological changes. There is found irregular acanthosis with thickening of the prickle cell layer, together with an irregular tendency to spongiosis of a very moderate degree, appearing, especially in the basal parts. In the cutis there is observed a slightly increased quantity of cells, consisting mainly of round cells but also of occasional polymorphonuclear leukocytes. These latter tend to migrate up into the epidermis. Some of the capillaries are slightly dilated (Plate 3). A similar histological picture is found after chromium treatment which has caused diffuse, irregular reddening. The increased quantity of cells in the cutis is then diffusely distributed without relation to the

Plate 1. Guinea pig treated with 0.0087 M La 8 Et during 10 days. No macroscopically visible changes. (Magnification 100 X.)

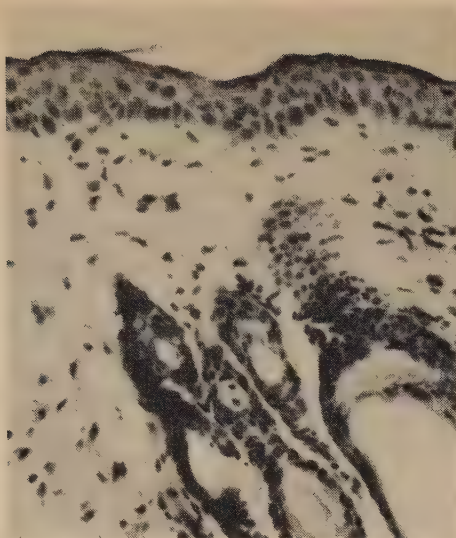
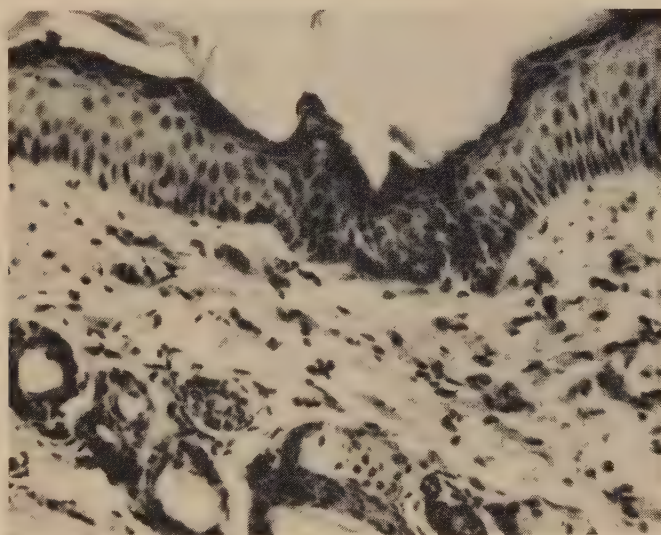


Plate 2. Guinea pig treated with 0.0347 M La 8 Et during 10 days. Macroscopic reddening with slight scaling. (Magnification 100 X.)



epidermal appendages. They occur, rather, in connection with the epidermal changes. The acanthosis and spongiosis described above appear more regularly when the skin exhibits an even reddening. The spongiosis can be very marked and tends to develop into vesiculation, while at the same time the macroscopic changes become more pronounced, with reddening, infiltration and desquamation (Plate 4).

Guinea pigs in a control group were treated with distilled water for 10 days in a uniform way. No macroscopic skin reactions arose, but histologically it was possible to show a moderately pronounced, irregular acanthosis (Plate 5).

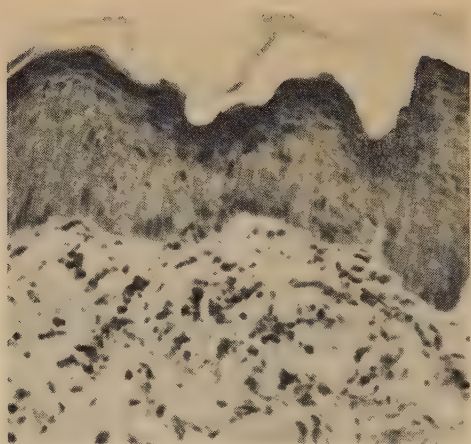


Plate 3. Guinea pig treated with 0.0174 M potassium bichromate for 10 days. No macroscopically visible changes. (Magnification 100 X.)



Plate 4. Guinea pig treated with 0.0174 M potassium bichromate during 10 days. Macroscopically visible, evenly distributed reddening with moderate infiltration. (Magnification 100 X.)

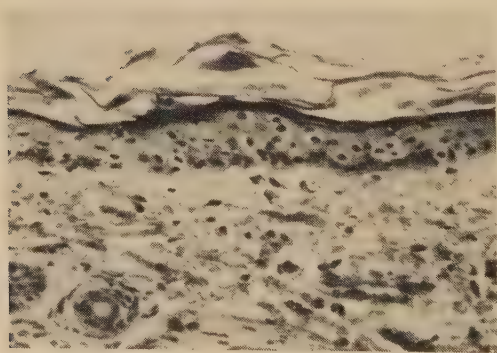


Plate 5. Guinea-pig skin treated with distilled water for 10 days. No macroscopically visible changes. (Magnification 100 X.)

Statistical analysis

In this processing of the results of the treatment the animals have been divided into those *with* and those *without* positive skin changes. A positive skin change is considered to be constituted by an evenly distributed reddening, with or without infiltration. In the latter group there have been included, in addition to the animals without macroscopically observable changes, those with diffuse, irregular reddening.

Table 18 shows the results after 5, 7 and 10 days' treatment in each of the 9 experimental occasions on which the guinea pigs were treated with chromium with and without La8Et. The statistical significance has been calculated according to the χ^2 test, and from Fisher's table there was obtained the value for p = probability that chance is the cause of the difference in the results for the respective groups.

In the same way there has been a processing of the results which appeared at treatment with the mixture of potassium bichromate and La8Et and with La8Et alone (Table 23).

If, as is the case here, the number of degrees of freedom is 1, χ is normally distributed with the mean value 0 and standard deviation 1, and then the total χ , with the retained sign, is also normally distributed. Although occasional χ -values are not exactly normally distributed, the total of them will be approximately so, if the number of χ -values is fairly large. — If the total of normally distributed χ -values is divided by the square root of the number of these, there is obtained the value for a normally distributed variable with a mean value 0 and standard deviation 1 = t . A table of the normal distribution then gives, finally, significance as regards the combined results after 5, 7 and 10 days' treatment. The following definitions of the significance have been used:

—	5	%	$\leq p$	:	no significant difference
*	1	%	$\leq p < 5$ %	:	almost significant difference
**	0,1	%	$\leq p < 1$ %	:	significant difference
***			$p < 0,1$ %	:	highly significant difference

At treatment with the two chromium solutions there appeared a significant difference after 5 days in only one of the experimental series (Table 18). This persisted during the whole period of the experiment. After 7 and 10 days there was a further series where the difference in the result was significant. On these two occasions, however, it was in different series that this appeared. — The number of animals in the different groups is relatively small, and therefore the χ -value is not very conclusive. A similar tendency, however, is found in all the experiments and in the summary analysis becomes more obvious. After 5 days' treatment there is an almost significant difference. On the 8th and 11th day this is strongly significant.

In Table 23 we find similarly that the difference in results which appears between animals treated with La8Et and with La8Et together with potassium bichromate has a similar tendency in the three experiments. This difference, however, is not always significant in the individual experiments. In the summaries showing the position after 5, 7 and 10 days' treatment the difference, however, is highly significant. It is calculated according to the χ^2 test and it is known already in advance in which direction the difference will be.

Discussion

So far as possible the experiments have been standardized in order to avoid exogenous and endogenous factors which may very well be supposed to affect the results. Thus the diet and general care of the animals were uniform. Similarly, the experiments were carried out during the winter months. The greatest difficulties were those connected with procuring the guinea pigs. This meant that on 2 occasions some animals had to be purchased from other sources than the regular supplier. Apart from this, other animals were sometimes of uneven quality to the extent that their weight varied by up to 150 grams, and moreover the animals appeared to react unfavourably with temporary loss of weight when put on a new diet. Whether the majority of the animals came from the same inbred strain cannot be with certainty established. The factors just mentioned may be partly responsible for the different results which appear when the animals are subjected to identical treatment. By identical treatment is meant that the animals were treated by a small number of persons who were familiar with the technical procedure. The status of the skin was inspected by two persons simultaneously and this was preferably done at midday in order to get as a uniform a lighting as possible.

The first macroscopically observable reactions were often expressed as an evenly distributed reddening. Often, however, there was found a more diffuse, unevenly distributed reddening which sometimes entirely disappeared but which, on other occasions, might remain for the whole period of the experiment. It was, however, more common for this diffuse, uneven reddening to become more evenly distributed during the course of the treatment and more obvious macroscopic skin changes occurred. It may seem doubtful whether this diffuse reddening should be regarded as a positive reaction arising as a result of the treatment of the skin with potassium bichromate. It was so uncertain in its course that it could be regarded as a reflection of an error of method. The possibility of a traumatization of the skin cannot be excluded. Several of the animals were in the cages together and it may easily have happened that some of them got scratched. The clipping also may be assumed to have caused a slight traumatization of the skin. In this work this skin change will *not* be regarded as positive, for the following reasons:

(a) The question investigated by this work is whether the ability of a substance to produce dermatitis on epidermal application can be increased by an addition of a surface-active non-protein-denaturing substance (p. 10).

(b) According to Table 16 reactions of strength (+) occur in 16 guinea pigs after treatment with the combination of potassium bichromate and La8Et whereas among the animals treated with potassium bichromate alone the corresponding figure is 9.

(c) If these reactions are now regarded as negative this cannot affect the evaluation of the results in a positive direction with respect to the question mentioned above.

The potassium bichromate solution used in the author's experiments, 0.0174 M, is one of about 0.5 %. Repeated daily painting with this on guinea-pig skin may result in local dermatitis in varying intensity. The reactivity of the guinea pigs seems to vary from experiment to experiment. In many groups no macroscopic skin changes occur (Tables 8, 9, 10, 13 and 15). In other experiments a number of guinea pigs reacted to the same treatment with dermatitis (Tables 11 and 12). These dermatitis appear at earliest after 7 days'

painting. This latency time tends to be shortened although the variations appear from experiment to experiment when the chromium solution also contains La8Et. The skin changes may then become manifest after only 5 days' treatment. At the same time as this the dermatites become more pronounced and the number of animals with positive reactions increases. The difference in results which appears at treatment with these two chromium solutions is not always significant in the individual series of experiments. A contributory reason to this is probably the fact that each experimental group consists of a relatively small number of animals. Sometimes the guinea pigs seem to be more reactive than at other times. When this is the case a difference established after 7 days' treatment may appear, but not after 10 days' painting. This is a consequence of the fact that the difference in the results between the groups become more and more levelled out the longer the animals have been treated. In the summaries showing the position after 7 and 10 days' treatment, however, there appears a statistically established difference between animals treated with potassium bichromate and animals treated with potassium bichromate together with La8Et.

As already mentioned, the first positive skin reactions may appear after 5 or 7 days' treatment respectively with the two chromium solutions. This long latency time supports the hypothesis that a non-direct primary irritation exists. On the other hand, dermatites can be developed in this way if the skin is exposed to a cumulative irritant substance, as can also happen at an epidermal sensitization. It is, however, not possible to distinguish, judging by this course, between two such phases, not least because many sensitizers moreover have a primary irritant effect.

In earlier experiments where guinea pigs were treated with potassium bichromate alone, no skin changes occurred (*Nilzén & Wikström, 1955; Samitz & Pomerantz, 1958*). An addition of SDS led to dermatites in all animals treated with it. The respective concentrations did not differ noticeably from those just described in the author's own studies. The number of animals in each group treated was, however, so small, being 2—5 in all, that no comparison between these results and the author's can be made. This also applies to other investigations in which chromium was applied epidermally to guinea pigs in different ways. Moreover, it is often not stated whether the animals got dermatites and in that case how many (*Mayer & Jaconia, 1958; Levin, Brunner & Rattner, 1959; Hunziker, 1960*).

The nonionic surface-active agent La8Et proved to be able to potentiate the ability of potassium bichromate to give rise at epidermal applications to dermatites. The detailed effect of La8Et is not clear. The same effect has been ascribed earlier to SDS and DDBS (*Nilzén & Wikström, 1955; Samitz & Pomerantz, 1958; Vinson, 1959*) in experiments with nickel sulphate as well as with potassium bichromate, whereas other surface-active substances did not show such tendencies (*Vinson, 1959*).

On the occasions when *Vinson* obtained dermatites with a mixture of nickel and a surface-active agent, he found nickel in the skin, especially in its appendages. No such deep penetration of nickel into the skin could be shown where corresponding treatment did not lead to any dermatites. As the detergents all have a surface-tension-reducing effect it is probable that other factors than this also contribute to the occurrence of nickel dermatites. In sufficiently strong concentrations these surface-active agents may have an ir-

ritant effect on the skin and give rise to acanthosis. *Baer* (1957) and later *Vinson* also pointed to acanthosis as such a contributory factor. Other irritations of the skin, for example traumatization with sandpaper, may also produce an acanthosis and a skin so treated is regarded as being easier to sensitize than a normal skin (*Rockwell*, 1955). — In the author's own investigations, it is true, it has proved that painting with La 8 Et can lead to an acanthosis. This is not so pronounced as it may be after treatment with potassium bichromate alone. It is therefore not probable that La 8 Et affects the development of chromium dermatites by its ability to give rise to an acanthosis. — Although there exists a certain difference with respect to acanthosis-promoting effect between the different substances, this does not exclude the possibility that the technical procedure is partly responsible for the occurrence of such skin changes. The gentle treatment of guinea-pig skin by means of a glass rod in order to distribute distilled water in a similar way can produce slight microscopic changes of the same kind.

— Sodium dodecyl sulphate has a strong protein-denaturing capacity and may consequently also affect the keratin (*Putnam*, 1948). The possibility that such a mechanism may promote the penetration of the chemical substance into the skin has been dealt with earlier (*Nilzén & Wikström*, 1955). — In the author's own experiments for this study the surface-active substance La8Et was used. This is nonionic in type and the concentration used, 0.0087 M, which corresponds to approximately a 0.5 % solution, gives the chromium solution a maximum reduction of surface-tension relative to water. La8Et has no known protein-denaturing capacity. In strong concentrations it is irritant to the skin. As used in the author's experiments it may be supposed that La8Et causes an acanthosis. This factor, possibly in combination with the surface-tension-reducing effect, would facilitate a penetration of chromium into the skin, with a consequent dermatitis.

In this connection it may be of interest to mention the effect of surface-active substances in animal experiments to induce skin cancer (*Setälä et al.* 1959; *Holsti*, 1959). Certain detergents were inactive in this connection, whereas others facilitated the appearance of a cancer when the skin was treated with a cancerogenous substance such as 9, 10-dimethyl-1,2-benzanthracene and 20-methylcholantrene. In such experiments it seems that different detergents may be more or less effective. Thus substances, such as Tween 60 and Tween 80, had a pronounced tumour-promoting effect, whereas Span 80 had a very weak activity of this kind.

Summary

A condensed account of epidermal sensitization of guinea pigs with simple chemical substances is given. A more detailed account of such experiments with chromium and nickel salts is given.

The author's investigations were carried out with potassium bichromate and the non-ionic surface-active substance La8Et, which is a lauryl alcohol to which has been added 8 moles ethyleneoxide. These two substances in high concentrations proved to be primarily irritating at epidermal treatment of guinea-pig skin. Solutions which contain 0.0174 M potassium bichromate and 0.0087 M La8Et did not produce a primary irritation.

A 0.0087 M La8Et solution applied daily to the skins of guinea pigs during 10 days did not produce any macroscopically observable skin changes. Treatment with 0.0174 M potassium bichromate solution in a similar way may after 7—10 days produce dermatites of varying strength. Such a potassium bichromate solution which also contains La8Et 0.0087 M can produce dermatites as early as 5 days after the beginning of the treatment. These dermatites have a tendency to be more pronounced than after treatment with potassium bichromate 0.0174 M alone.

Treatment with potassium bichromate in combination with La8Et leads to dermatites in more guinea pigs than is the case at treatment with potassium bichromate alone. The difference which appears between these two groups is statistically significant after 7 and 10 days' treatment.

The effect of surface-active substances on epidermal applications of different simple chemical substances is discussed.

CHAPTER III

Do the reactions which arise after repeated applications on the epidermis with potassium bichromate solutions reflect a primary irritation and/or a condition of hypersensitivity?

The epidermal treatment of guinea pigs with 0.0174 M potassium bichromate in water solution caused local dermatites in some of the animals. The capacity of potassium bichromate to produce skin changes in this way increased as a result of the addition in a chromium salt solution of a nonionic surface-active agent, La8Et, in the concentration 0.0087 M. The first macroscopically observable skin changes could appear at earliest after 5 days' treatment and usually these dermatites increased in intensity with continued exposure to chromium. The course now described is such that the skin changes can be supposed to be an expression of a hypersensitivity caused through a sensitization. It proved, however, that chromium in a strong concentration, e.g. 0.174 M, may have a primarily irritant effect on the skin of the guinea pig and cause fully observable reddening after 1—2 day's treatment (p. 13). It can therefore be presumed that a weaker chromium solution such as 0.0174 M can also have a primarily irritant effect on the skin. The skin change arising in this way is, however, not the result of an immediately irritant effect but rather the consequence of an irritation with cumulative effect. Dermatites of this kind appear after approximately the same latency time as the hypersensitivity reactions referred to above. One further possibility may however be conceived of; parallel with the development of a dermatitis on the basis of a cumulatively irritant effect of chromium, an epidermal hypersensitivity to chromium may have arisen.

In order to investigate more closely whether the epidermal treatment of guinea pigs with potassium bichromate caused a primary irritation and/or a hypersensitivity to chromium, the animals will be tested. Experiments on passive transfer of such a hypersensitivity through parabiosis will also be carried out as a complement to the testing.

Generally speaking, an epidermal hypersensitivity is best obtained through epidermal treatments with or through intracutaneous injections of a sensitizer (*Landsteiner & Chase*, 1940; *Chase*, 1954). A sensitivity of contact dermatitis type may, however, also arise after intramuscular injections of a w/o emulsion containing an allergen in water phase and complete Freund's adjuvant (Difco)®. As a result of this treatment the skin sensitivity may be very intensive. It was therefore considered to be of great value to sensitize guinea pigs with potassium bichromate in this way. The expression of a chromium hypersensitivity which appears as a result of testing and parabiosis was also investigated on these guinea pigs.

A. Testing of guinea pigs exposed to chromium sensitization

Material and Methods

Experimental Animals. The epidermal pretreatment was carried out on albino female guinea pigs weighing between 200—400 gm. Most of these animals had come from the same supplier (p. 11) who supplied albino female guinea pigs in similar circumstances for the experiments with chromium and Freund's adjuvant. The diet of the animals and the procedure have also been described earlier p. 00.

Technique of Sensitization. The epidermal pretreatment of the guinea pigs with potassium bichromate with or without addition of La8Et is described on p. 12.

For sensitization with chromium and Freund's adjuvant a w/o emulsion was prepared. For this purpose 10 ml of a 0.0174 M potassium bichromate water solution (glass-distilled) was mixed with 10 ml complete Freund's adjuvant (Difco). The fact the w/o emulsion had really been obtained was verified by microscopy. On alternate days, on three occasions in all, 0.2 ml of this emulsion was injected in one of the animal's hind legs. In this way each animal obtained in all approximately 1.5 mg potassium bichromate.

Technique of testing. The guinea pigs which had been pretreated epidermally with potassium bichromate with or without addition of La8Et were tested with chromium 14 days after the commencement of primary chromium treatment. Testing with La8Et was carried out on animals which 5—6 weeks earlier had been subjected to the primary treatment with chromium + La8Et and which 2 weeks after that treatment were also tested with chromium. This meant that La8Et was applied to skin which had earlier been treated with chromium. At the same time the guinea pigs were tested on the contralateral side with the mixture of chromium and detergent. These skins also had been treated with chromium earlier.

The guinea pigs which had been pretreated with intramuscular injections of chromium + Freund's adjuvant were tested with chromium 3 weeks after the commencement of sensitization.

Test solutions consisted of potassium bichromate 0.0174 M and La8Et 0.0087 M. The solvent was glass-distilled water.

At testing during 2 minutes 0.2 ml of the test solution was massaged with slight pressure by means of a glass rod over the skin surface which had been clipped with an electric clipper. This surface had not previously been exposed to chromium treatment. As regards La8Et, see above. The treatment was repeated daily for three days.

Reading for possible skin reactions was undertaken at each test application and 24 hours after the last application. The status of the skin is stated as follows:

- 0 no macroscopically observable reaction
- (+) diffuse, irregularly distributed reddening
- + fully observable reddening over the greater part of the treated skin surface
- ++ reddening with infiltration.

Results of Testing with Potassium bichromate and La8Et.

In Table 24 are shown the results for 27 guinea pigs which had previously been treated epidermally with a 0.0174 M potassium bichromate solution in order to obtain a sensitization. Of these animals one became emaciated and had to be destroyed. In 7 of the remaining guinea pigs there arose skin changes which were considered to be positive. These appeared at earliest after 8 day's treatment. At testing, 6 guinea pigs reacted with reddening after 3 days' treatment. None of the animals which had not obtained dermatites during the primary 10 days' treatment reacted to the ensuing testing.

In table 25 are reported 61 guinea pigs which had been pretreated epidermally with potassium bichromate in combination with La8Et. As one of the animals became more and more emaciated without visible signs of disease it was destroyed after five days' treatment. Some of the other guinea pigs reacted with skin changes, which appeared at earliest after 5 days' repeated applications of chromium-detergent mixture. During the ensuing days a number of animals with positive reactions increased and after 10 days a total of 42 guinea pigs had obtained dermatites. The majority of these dermatites became more and more pronounced as the treatment proceeded. — At testing on the contralateral side with potassium bichromate such an individual disposition to react again appeared in the guinea pigs. Some of the animals remained without skin changes whereas others had reactions, the intensity of which varied. Practically all these guinea pigs were the same animals that obtained dermatites at the primary treatment with chromium in combination with La8Et. There were some exceptions: animal No. 0 in the experiment of 11/22/60 and animal No. 32 in the experiment of 12/5/60. These guinea pigs had no skin changes at their first treatment with chromium but they had skin changes at the testing. The opposite situation also occurred, namely that the guinea pigs reacted positively to the primary treatment with a combined chromium-detergent solution, but at the testing no reactions appeared, (e.g., experiments of 11/24/58, 11/23/59). — The positive testreactions appeared at earliest during the second day of the testing. Then 4 of the total of 60 guinea pigs had obvious reddening and the number of animals with positive reactions increased as the testing continued. After 3 days' treatment 17 guinea pigs had reddening.

From Table 26 it will be seen that none of the 19 animals which had been tested with La8Et obtained macroscopically observable skin changes with this substance. On the other hand, during the same period a number of guinea pigs showed positive test reactions to the mixture chromium-La8Et. Some of these reactions appeared as soon as 2 days after the commencement of testing.

In all 59 guinea pigs had intramuscular injections of potassium bichromate together with Freund's adjuvant and during this treatment 2 animals showed increasing lack of appetite and emaciation. They were therefore destroyed. No other signs of disease were noted on section. The results of the ensuing testing are shown in Table 27. In all 27 guinea pigs had positive test reactions. Of these 3 were manifest after 24 hours but the majority appeared after a further 24 hours. It was found that the skin changes varied in intensity from animal to animal. Even in the same guinea pig the intensity of the reaction might vary from day to day and usually an evenly distributed reddening developed into a reddening with infiltration. The opposite state of affairs was, however, found; animal No. 25 in

the experiment of 10/6/61 showed after 2 days' treatment an evenly distributed reddening. One day later this reaction became weaker and could then no longer be counted as positive.

Discussion

Potassium bichromate has in a high degree a primarily irritant effect on skin. A concentration of 50 microgram/ml produces a pronounced epidermis damage in *in vitro* experiments on human skin (Hu *et al.*, 1956). *In vivo* the skin tolerates stronger concentrations, e.g. 0.5 %, without macroscopic signs of damage appearing (Rostenberg & Sulzberger, 1939). Corresponding data as regards experimental animals such as guinea pigs do not exist but potassium bichromate in the concentration 0.174 M (which corresponds approximately to a 5 % solution) has proved capable of producing skin changes of primarily irritant type, according to earlier investigations by the present author (see p. 13). With the concentration used in these experiment, 0.0174 M, with or without addition of La8Et, dermatites were produced in some animals. Other guinea pigs, on the other hand, did not react with macroscopic skin changes. At testing with chromium solution on the contralateral side such an individual disposition to react again appears in the guinea pigs. Here there is a tendency for the same animal to react similarly from one occasion to another. Such variations between animals exposed to the same treatment under identical conditions are considered to be genetically conditioned and had been noted earlier by *inter alios* Chase (1941). He showed that this was the case on sensitization but also as regards the occurrence of primarily toxic skin changes.

The reactions which appear at chromium testing (stage 2), appear broadly speaking after a shorter latency time than in the earlier chromium treatment (stage 1). This could be explained by the skin changes, which had already occurred in the experimental animal, having led to a generally increased irritability of the skin. This latter disposition to react is then to be regarded as unspecific and the essential factor is the primarily irritant property of the chromium. By this is meant that the animals' irritation threshold to such influence has been lowered. If that was the case, a logical consequence would be that the irritation threshold of the animal for other primarily irritant substances would also be lowered. Such a substance is, for example, the surface-active agent La8Et which in stronger concentrations is markedly primarily irritant on guinea pig skin (page 12). In the concentration used 0.0087 M, no increased reactivity to this appeared at testing, however. At the same time the combination chromium-detergent caused in the same animals dermatites as early as 2 days after the commencement of treatment (Table 26). Such a reaction mechanism with reduced irritation threshold has been dealt with by, among other authors, Rostenberg (1957) as regards man, but of greater interest in this connection are the animal experiments with dinitrochlorobenzene (DNB) of Chase (1941). He showed that no parallelism exists between the sensitizing ability of a substance and its primarily irritant effect on the skin and that a poor reactivity to picrylchloride in an animal is not affected by sensitizing it to DNB. The animals were tested with both substances at the same time. Similar results were reached by Ginsberg *et al.* (1937). These authors also treated one group of guinea pigs with poison ivy extract and another group with phenol solution. The

latter caused local dermatites. Untreated guinea pigs in a third group were used as controls. At testing with poison ivy no difference appeared between the phenol-treated guinea pigs and the control animals, whereas an increased reactivity could be shown in those which had been previously painted with poison ivy. Thus these authors clearly show that a shortened latency time at testing with a substance is not related to a previously provoked purely primary toxic irritation. Rather, the shortened latency time at testing is a consequence of a hypersensitivity to the test substance in question. The testing carried out by *Ginsberg et al.* took place 4 weeks after the animals had been subjected to the phenol treatment. On the other hand, *Haerberling & Fox* (1959) have shown that the skin of a guinea pig may be generally more irritable when a toxic dermatitis has reached its maximum. In the present author's investigations however, the primary dermatitis had decreased when the testing was carried out; moreover, a shortened latency time could also be shown on treatment with chromium + La8Et 4—5 weeks after the primary dermatitis was at its most pronounced stage.

The latency time stands in a certain relation to the degree of sensitivity reached and to the concentration of the sensitizer. It is stated to be 20—48 hours (*Chase* 1954). A shorter latency time is associated with a more pronounced status of "hypersensitivity" than is a longer latency time (*Nilzén*, 1952). The degree of sensitivity may vary with different substances; some substances can give rise to a very pronounced sensitivity, other substances much less so. It may also vary from one animal to another exposed to treatment with the same substance (*Bloch, Steiner-Wourlich*, 1930). At testing with potassium bichromate it is found that the time until the reactions become manifest varies. The first reactions come between 24 and 48 hours after the commencement of treatment. Within 72 hours 17 of the 60 animals reacted positively (Table 25). This frequency, like the latency time, differs markedly from what is normal in experiments with substances with a strong sensitizing capacity such as DNB, primulin etc. In comparisons of this kind it should however, be borne in mind that the solvent of the substance in question may affect the results. *Nilzén* (1952) also emphasizes this. Vehicles such as alcohol and oil (the most common solvents for DNB) are considered to promote the occurrence of such reactions in relation to water (in which the potassium bichromate is dissolved).

At the first treatments of the guinea pigs with chromium-detergent solution the first skin changes appeared, as already mentioned, after 5 days' treatment. At that time 7 animals had dermatites, and these became more and more pronounced as the treatment continued. The course agrees well with what is seen in a DNB sensitization and in analogy with this it could be assumed that a chromium sensitivity had been developed. When the animals were tested later there appeared positive test reactions within 3 days in some of these guinea pigs but in other guinea pigs no positive test reaction appeared (Table 25). In the latter case it is doubtful whether a sensitization to chromium took place when the guinea pigs were subjected to the primary chromium treatment. The dermatites which arose in connection with this might be the expression of a primary irritation with cumulative effect. Similar modes of reaction however, have been observed also in established DNB sensitization (*Frey*, 1951). It might happen that sensitized animals suddenly did not react to test concentrations, which in ordinary cases, moreover, were primarily irritant. *Frey* says that the animals are hyposensitive = "unterempfindlich".

— The opposite state of affairs is also found. Guinea pigs which react little or not at all to the primary chromium treatment have positive test reactions (Table 25, animal 0 in the experiment of 11/22/60, 32 in the experiment of 12/5/60). Such a sensitization course has earlier been dealt with by *Landsteiner & Jacobs* (1935).

Animal experimental studies of the type now described have hitherto mostly been carried out with compounds such as picryl chloride, DNB and substances closely related with these. As a rule only one challenging dose of the allergen has been given, this proving sufficient to obtain positive test reactions. In similar experiments with chromium some authors have tested the animals once (*Nilzén & Wikström*, 1955; *Samitz & Pomerantz*, 1958 and *Mayer & Jaconia*, 1958). Other authors have not stated the number of challenging doses (*Levin, Brunner and Ratner*, 1959; *Hunziker*, 1960). With potassium bichromate *Nilzén & Wikström* obtained positive test reactions within 24 hours. In the present investigations, after pretreatment with intramuscular injections of chromium + Freund's adjuvant, positive test reactions appeared on some occasions within 24 hours, i.e. after a single chromium treatment (Table 27). The majority of positive test reactions in these guinea pigs, however, appeared only after a further painting with chromium, or within 48 hours. This time interval agrees well with that given by *Chase*, 20—48 hours, (1954). On no occasion were such skin changes provoked so rapidly when the guinea pigs were treated for the first time epidermally with 0.0174 M potassium bichromate solution with or without addition of the nonionic surface-active agent La8Et. The manner in which the skin reacts to chromium after preceding intramuscular injections of chromium + Freund's adjuvant argues in favour of the hypothesis that an epidermal hypersensitivity to chromium has arisen. After testing for 3 days such a reaction could be shown in 27 of the 57 surviving guinea pigs, i.e. in rather fewer than 50 % of all the animals. The corresponding figure after epidermal pretreatment with potassium bichromate + La8Et was 17 of 60 surviving guinea pigs, i.e. fewer than 30 % had positive test reaction. The difference is significant with an χ^2 -value of 5.682 on a comparison between the two treatment groups as a whole. There is another difference also. Positive test reactions may occur within 24 hours in animals which have been pretreated with chromium + Freund's adjuvant. In the present series of the experiments this did not succeed in any of the guinea pigs which had earlier had epidermal treatment with chromium-detergent mixture. In these animals there seems to be a time lag as regards the development of the test reactions. This, however, is not a statistically established difference, and the increased reactivity in the three guinea pigs which at testing received dermatitis within 24 hours may also be supposed to be genetically conditioned. In similar experiments *Hunziker* (1960) also did not get results which could show that an epidermal sensitivity to chromium was more intensive after injections of chromium in combination with Freund's adjuvant than what could be caused by an epidermal chromium treatment (Table 2). With the former method 7 of 15 guinea pigs became sensitive to chromium, whereas no positive test reactions occurred in any of the 9 animals pretreated epidermally. These experiments, however, cannot be compared with the present author's, as in *Hunziker's* work there is no statement of the number of test applications or of the time which elapsed from the commencement of testing until positive skin reactions appeared.

Summary

(1) Guinea pigs were treated epidermally with potassium bichromate with or without addition of the nonionic surface-active agent La8Et. Dermatitis developed in some of the guinea pigs. At the same time the sensitivity to chromium of the animal's skin changed. The test results support the hypotheses that after the occurrence of the primary dermatitis a hypersensitivity to chromium can be shown.

(2) Guinea pigs which had previously been treated with intramuscular injections of potassium bichromate + Freund's adjuvant were tested epidermally with potassium bichromate water solution. In a number of guinea pigs the skin reacted to this treatment with reddening, with or without oedema. These skin changes developed more rapidly than is the case on corresponding treatment of normal animals. The results support the hypothesis that guinea pigs can be made hypersensitive to chromium in this way.

(3) Treatment of guinea pigs with potassium bichromate + Freund's adjuvant leads to a more frequent epidermal sensitivity to chromium than does epidermal pretreatment with potassium bichromate + the nonionic surface-active agent La8Et. No certain difference appears as regards the intensity of the epidermal sensitivity to chromium as between the two treatment groups.

B. Cutaneous Parabiosis

In the results reported above we showed that epidermal treatments with potassium bichromate or intramuscular injections with potassium bichromate + Freund's adjuvant could bring about an increased skin reactivity to chromium in a manner which supported the hypothesis that a sensitization had taken place. Experiments to transfer passively such a sensitivity to a normal animal have been carried out through cutaneous parabiosis. The method as such is not new. The first researcher to concern himself with it seems to be *Paul Bert*, 1862 (quoted from *Finerty*, 1952). After this various authors have used the method, with certain variations (*Sauerbruch & Heyde*, 1908; *Bunster & Meyer*, 1933; *Haxthausen*, 1944; *Skog*, 1955). The primary problem here has been whether vascular connections arose between donor and recipient. Histologically *Sauerbruch & Heyde* have indicated the occurrence of such connections, and functionally *Van Dyke, Huff & Evans* (1948) as well as other authors have demonstrated the same phenomenon.

Technique of Operation. As donors there were used both guinea pigs which had obtained pronounced dermatitis in the epidermal treatment with potassium bichromate + La8Et and guinea pigs which had been pretreated with intramuscular injections of potassium bichromate + Freund's adjuvant. The former were not tested after this first-stage treatment but the latter were tested 3 weeks after the commencement of sensitization. Only guinea pigs with positive test reactions were chosen as donors. The recipients were female guinea pigs of the same age.

The operation was carried out under anaesthesia. The animals were anaesthetized by intramuscular injections of Nembutal® each animal being given 0.02 g/kg body weight. — Along the sides which were to be joined an incision 7 cm long was made in the skin. In the muscles there was made a 5 cm long incision into the peritoneum which was left

undisturbed. Catgut No. 00 was used for the suturation. The muscles were sown first and then the skin. In this way the incision surfaces in the donor and recipient were made to lie against one another. In order to avoid too great a strain on the edges of the incision the animals were fixed together with adhesive tape 5 cm wide.

Technique of Testing. This was carried out postoperatively during the 4th—7th day. By means of a glass rod 0.2 ml of test solution was massaged with a slight pressure over the rear free flanks of the recipient and the donor for 2 minutes daily on 3 successive days. Reading for possible skin reactions was undertaken before each test application and 24 hours after the last one. The status of the skin was given as described on testing above (p. 23).

The test solution consisted of potassium bichromate 0.0174 M in combination with La8Et 0.0087 M.

Results. In all, 12 experiments were made with cutaneous parabiosis in which the donor was pretreated epidermally (Table 28). All animals survived the operation and 3 days' testing. A number of the guinea pigs, however, began to lose their appetite towards the end of the test period and became emaciated. No difference seemed to exist in this respect between donor and recipient. Similarly at this point there could be noted in some guinea pigs a tendency to partial separation of the sutured skin surfaces. Two of the donors reacted with reddening to the testing and on one of these occasions there appeared a similar reaction in the recipient. All these changes were fully evident within 72 hours.

From the guinea pigs which had been sensitized through injections of chromium and Freund's adjuvant 15 donors were chosen. These and the corresponding recipients survived the operation, but before the testing was completed two donors had become more and more emaciated and had to be destroyed (Table 29). This also occurred with one of the recipients. A number of other guinea pigs showed increasing loss of appetite and became emaciated. At the end of the test period a tendency to partial separation of the animals could be noted. Broadly speaking the recipients stood the treatment better than the donors. — Positive test reactions appeared in 4 donors and 4 recipients, but in only one of the experiments did the skin changes appear simultaneously in donor and recipient. In another experiment there occurred a positive reaction in the donor at the same time as a diffuse, unevenly distributed reddening appeared in the recipient. Of the donors, 2 had reddening without the corresponding recipient showing any tendency to reactivity. On the other hand, apart from the experiment already mentioned where donor and recipient reacted equally strongly, positive skin reactions in the recipients seemed to be associated with an increased reactivity to chromium in the respective donor. The skin changes in the donors which appeared in this connection were, however, not so pronounced that they could definitely be regarded as positive. They consisted of a diffuse, unevenly distributed reddening.

The positive test reactions could be manifest within 24 hours after the commencement of treatment. This occurred in the experiment where donor and recipient reacted in the similar way. During the ensuing day a further donor and 2 further recipients had achieved positive test reactions.

Discussion. A number of authors have earlier attempted to transfer by parabiosis an epidermal sensitivity to a normal animal. The agent mainly used is DNB, which proved

to possess a strong sensitizing capacity. This has not prevented different authors from having varying amounts of success in the attempt. Thus *Haxthausen* (1944) did not succeed with cutaneous parabiosis in the few experiments he carried out. At similar investigations *Skog* (1955), on the other hand, obtained positive results in a number of cases and the recipients' reactions could be as pronounced as those of the donors. *Skog* also pointed out that such parabiosis experiments with a strongly sensitizing substance usually succeed to not more than 60 %.

The ability of potassium bichromate, in the author's investigations, to give rise to an epidermal hypersensitivity in guinea pigs is inferior to that of DNB. This may probably explain the difference in the results between cutaneous parabiosis experiments with chromium dissolved in water and such experiments with DNB dissolved in alcohol or oil. In the former case the latency time is prolonged and any reactions appear at earliest 7 days after the operation. At that time there is a great risk of parabiosis intoxication (*Finerty et al.* 1951). In the present investigations it could also be established that one week after the operation the animal lost appetite and began to become emaciated. At the same time the sutured skin connections between the animals could break apart. It may therefore seem to be rather late to begin testing of the parabionts on the fourth day after the operation, but it is doubtful whether the test results can be affected through testing at an earlier stage. This also emerges from *Skog's* experiment with cutaneous parabiosis. He says: "In these experiments parabiosis for two and three days did not suffice to transfer DNB sensitivity. On the fourth day of parabiosis, however, there were distinct reactions . . .". — In order in some degree to try to facilitate the occurrence of positive test reactions to chromium in parabiosis experiments, there was used as test solution potassium bichromate in combination with the surface-active agent La8Et. Such a combination has proved, in the experiments reported above, to be more reactive than potassium bichromate alone. But although this mixed solution with chromium and detergent was applied epidermally on guinea pigs not previously treated with chromium, the first skin changes did not appear as early as after 3 days' treatment. It was not until the sixth day that any reactions became manifest. The reactions which appeared after 3 days' treatment in the parabionts must therefore be regarded as an expression of a hypersensitivity to chromium having arisen. The frequency of epidermal sensitivity to chromium transferred in this way is low, i.e. in one case out of 12. These figures, however, cannot be compared with the statement given earlier, that such cutaneous parabiosis experiments usually succeed up to not more than 60 %. At the primary treatment with chromium the donors, it is true, had clear dermatites, but before the operation it was not investigated by testing how many of these had obtained hypersensitivity to chromium. On the other hand, the guinea pigs could be tested postoperatively on a skin surface not previously treated with chromium. This was considered to be of great importance for getting a better evaluation of the results from testing of donors and recipients respectively. This does not however, necessarily mean that only 2 of the donors developed a hypersensitivity to chromium as result of the primary chromium treatment. As will be seen from Table 25, an increased epidermal skin sensitivity to chromium may be of varying intensity. If it is less pronounced there may be difficulty in showing it after the guinea pigs have been exposed to such an extensive operation as cutaneous parabiosis. On the other hand, a very pronounced

epidermal hypersensitivity to chromium must have been provoked as a result of the primary epidermal chromium treatment in the two donors which had positive test reactions. Injections of chromium together with complete Freund's adjuvant produce a more frequent sensitivity to chromium than an epidermal chromium treatment (p. 27). This also clearly appears from *Hunziker's* investigations (1960). As regards the intensity of the increased skin reactivity no difference appeared after treatment according to the two methods. Such a difference has, however, been shown in similar experiments with picrylchloride (*Landsteiner & Chase*, 1940). In the present author's experiments with epidermal sensitization of the donors, a positive test reaction appeared in only one recipient out of 12. After pretreatment with chromium and Freund's adjuvant on the other hand, 4 recipients had positive test reactions, and it is possible that this difference is due to the fact that a more intensive sensitivity to chromium arose in the donors as a result of treatment according to the latter method. Seeing that 4 out of 12 surviving donors had positive test reactions and the corresponding number after epidermal pretreatment was two out of 12, this also may argue in favour of the hypothesis that a more frequent hypersensitivity to chromium arises after intramuscular injections with potassium bichromate and Freund's adjuvant. This increased frequency, however, does not correspond to that which occurs at testing of guinea pigs pretreated in the same way but not subjected to the trauma which an operation in general and a cutaneous parabiosis in particular must involve. One week after such an operation there is a great risk of parabiosis intoxication, as *Skog* (1955), among other authors, has pointed out. Guinea pigs showed at this critical time signs of loss of appetite and emaciation. The donors seem generally to have been affected in a higher degree than the recipients. The reduced power of resistance of the donors at parabiosis may also be supposed to be a consequence of the intramuscular injections of the metal salt potassium bichromate. This is biochemically very active because of its oxidizing capacity and in all probability will be transported to various organs, with the risk of causing toxic damage to them. This may very well explain why on several occasions the recipients had stronger test reactions than the corresponding donors. The results show, broadly speaking, that injections of potassium bichromate together with Freund's adjuvant give a more intensive sensitivity to chromium than epidermal treatment of chromium with or without addition of the surface-active agent La8Et.

Summary

(1) Experiments to transfer by cutaneous parabiosis an epidermal sensitivity to chromium to guinea pigs not previously treated with chromium were carried out. The sensitivity to chromium was tested by epidermal testing. Signs of parabiosis intoxication appeared in the majority of the guinea pigs.

(2) After epidermal pretreatment of the donors a passive transfer succeeded in one experiment out of twelve.

(3) A sensitization through injections of potassium bichromate and Freund's adjuvant produces a sensitivity to chromium which was passively transferred to 4 of 12 surviving recipients.

CHAPTER IV

GENERAL DISCUSSION

Introduction

In 1955 Nilzén & Wikström published results from animal studies. They treated guinea pigs epidermally with potassium bichromate under the conditions of a two stage multiple application technique. Stage one (the sensitization period) involved treatment with a view to sensitizing the animal to the metal salt. During stage two (the testing period) the animals were again exposed to chromium. Nilzén & Wikström showed that epidermal treatment with potassium bichromate could lead to local dermatites in the presence of a surface-active agent, the anionic sodium dodecyl sulphate (SDS). On the other hand, the potassium bichromate solution in itself caused no macroscopically observable skin changes. During the ensuing testing period there appeared results which supported the hypothesis that a dermatitis obtained during stage one led to a markedly increased skin reactivity to chromium. The authors considered that a sensitization had arisen. The detailed effect of SDS was discussed and, among other things, its surface-active capacity and its effect on proteins were pointed out. In the present work the question is therefore posed whether the capacity of potassium bichromate to give rise to dermatites on epidermal application can be potentiated if the chromium solution contains a nonionic surface-active agent which has no protein-denaturing effect. It has further been investigated whether a dermatitis which has arisen in this way is the expression of the primary irritation by and/or a sensitization to potassium bichromate. The results of the studies have produced the following answers:

(1) Epidermal treatment of guinea pigs with a 0.0174 M potassium bichromate water solution may cause local dermatites. The presence of a lauryl alcohol-ethylene oxide condensate in the concentration 0.0087 M, here referred to as La8Et, leads to an increased frequency of such dermatites. The difference between the two groups of treatment is statistically significant.

(2) After the occurrence of these primary dermatites a hypersensitivity to chromium can be shown. This can be passively transferred to a normal animal through cutaneous parabiosis. These results agree well with those which have emerged from similar experiments where sensitization of guinea pigs has been effected through injections of potassium bichromate + complete Freund's adjuvant (Difco).®

It is well known that epidermal sensitization experiments are difficult to standardize. In my investigations this has largely succeeded, apart from the difficulty of obtaining guinea pigs from the same strain. This difficulty meant that the investigations had to be divided up into small series of experiments in order to get the animals in the two treatment groups as evenly distributed as possible. In all, 103 guinea pigs were treated with potassium bichromate solution and a similar number with the mixture of potassium bichromate and detergent.

Earlier studies within this field have given varying results (*Mayer & Jaconia*, 1958; *Samitz & Pomerantz*, 1958; *Levin, Brunner & Rattner*, 1959; *Hunziker*, 1960). In these studies, however, the number of experimental animals has been so small that the results cannot in detail be compared with the author's own. The surface-active substance in all the studies mentioned, with the exception of *Levin et al.*, was SDS.

Epidermal treatment with La8Et and/or potassium bichromate

The present author's investigations show clearly that in identical circumstances guinea pigs may react differently to the same treatment. *Chase* (1941) mentions that this may be the case in sensitizations as well as on primary irritations and the differences are probably genetically conditioned. Without reference to such variations there appears in the individual series of experiments during stage one a tendency to different results at treatment with chromium and chromium + La8Et respectively. Throughout, more guinea pigs reacted with positive skin changes to the chromium-detergent mixture than to the chromium solution alone. With the combined solution dermatitis may be manifest as early as after 5 days' treatment, whereas the chromium salt solution alone gives dermatitis at earliest after 7 days' treatment. Towards the end of the respective exposure to chromium the number of animals with positive skin changes in the individual series of experiments may to a certain extent tend to level out. This supports the hypothesis that the surface-active substance, apart from contributing the occurrence of a more frequent appearance of chromium dermatitis, also hastens on the development of this dermatitis. A comparison in a wider sense shows that such a frequency increase as now described may also occur on the addition of detergent to a nickel sulphate solution in similar experiments (*Vinson*, 1959). *Vinson* found that a difference in this respect appeared according to what surface-active substance was used. Certain anionic detergents such as SDS and sodium dodecyl benzene sulphonate were most active. Other anionic and also nonionic substances tested by *Vinson* had little or no effect. When dermatitis arose, nickel could be demonstrated in the dermis, the pilosebaceous apparatus and the basal layer of the epidermis and *Vinson* considered that the acanthotic effect of the respective substances was of greater importance than the surface-active effect in this connection. La8Et, too, in stronger concentration had a pronounced acanthotic effect, but in the concentration 0.0087 M this is not so marked. It is, moreover, inferior to that possessed by potassium bichromate in the concentration 0.0174 M without addition of detergents, although no macroscopic skin changes appear. It therefore appears most probable that an acanthosis, independently of what irritation has caused it, involves a weakening of the defence for skin against the penetration of substances which can induce dermatitis. Penetration into the skin of such substances in water solution can be facilitated through the addition of surface-active substances, as has been shown by *McKee et al.* (1945). The surface-active agent La8Et in the concentration 0.0087 M reduces the surface tension for the potassium bichromate solution to half (relative to water). Such a reduction of the surface tension will probably give a satisfactory effect in this respect (page 11).

Testing after epidermal pretreatment with potassium bichromate

In connection with the dermatites appearing during stage one in the present author's investigations, it was found that an increased skin reactivity to chromium might appear. This expressed itself in a shortened latency time for the appearance of dermatites on a renewed exposure to chromium during stage two treatment. These results agree with those which were obtained by Nilzén & Wikström (1955) with chromium and SDS and which Levin *et al.* (1959) later also confirmed. Mayer & Jaconia (1958), on the other hand, did not succeed in showing such an increased skin reactivity to chromium after preceding epidermal chromium treatment. This was carried out, however, every other day, in all three times. Of greater interest are the experiments carried out by Hunziker (1960) with epidermal chromium sensitization *ad modum* Nilzén & Wikström (1955). After 8 days' treatment the authors were unable to show any positive epicutaneous test in the 9 experimental animals. It does not, however, appear from the article whether the sensitization treatment caused dermatites. On the other hand, Samitz & Pomerantz (1958) induced a dermatitis in 4 experimental animals but they were unable to show any increased skin reactivity to chromium on testing. This was carried out fully three weeks later. The experiments of different authors as described above have, however, been carried out on a limited scale and therefore among other things the results cannot unfortunately be compared with the experiments in the present work.

The primary chromium dermatites reported in this work were developed in such a way that they could be assumed to be the expression of a sensitization to chromium or an irritation caused by a cumulative effect of the chromium treatment. It seems probable that both these phenomena may occur at the same time. Against the sensitization the circumstance can be argued that the dermatites may appear after 5 days' treatment with the mixture of potassium bichromate and La8Et. It is probable that no sensitization to chromium has become manifest as quickly as this. It would rather seem that at this point an irritation of the skin exists. This does not exclude the possibility that a sensitization is in progress. It is known that many of the substances which can produce an epidermal hypersensitivity can also primarily irritate the skin in certain concentration. This may naturally also apply to potassium bichromate. In support of this may be adduced the short latency time for the appearance of new dermatites during stage two. Certain reservations may, however, be made. Rostenberg (1957) has pointed out as regards man the possibility that a primary irritation can generally reduce the irritation threshold for other irritant substances. He considers that a polyvalent, increased reactivity arises. Experimentally Haeberling & Fox (1959) have shown that when a skin reaction induced by 40 % crotonoil has reached its maximum, normal skin in the animal seems to be more receptive to primary irritation than ordinarily. If a generally reduced irritation threshold in the author's experiments existed, however, then in the light of what has been mentioned above (Haeberling & Fox 1959), more guinea pigs which had developed primary dermatites ought to have reacted with skin changes at the testing. Moreover, this testing was not carried out when the primary dermatitis was at its maximum, and on the occasions when testing was carried out 5—6 weeks after the beginning of sensitization a shortened latency time nevertheless occurred (page 24). Against the assumption that a dermatitis

through primary irritation would generally enhance the disposition to reaction there may be cited also investigations by *Ginsberg et al.* (1937). These authors found that an earlier toxic dermatitis provoked with phenol did not change the irritation threshold of the animals to poison ivy extract in the dilution 1/10 as compared with control animals. They came to the conclusion that a shortened latency time was the consequence of a sensitization which had arisen earlier.

The increased skin reactivity to chromium which may arise after earlier epidermal chromium treatment is expressed in the same way as the corresponding phenomenon after pretreatment with potassium bichromate in combination with Freund's adjuvant. With the latter method, however, comparatively more guinea pigs with positive test reactions are obtained. An increased frequency of chromium sensitivity was also obtained by *Hunziker* (1960) after injections of potassium bichromate together with complete Freund's adjuvant. Positive chromium tests occurred epidermally as well as intracutaneously. The latter testing method gave more positive reactions than the epidermal one. According to *Hunziker*, this is to be explained by the fact that in eczema there may be two shock organs, the dermis and the epidermis, and these do not always respond simultaneously at testing. *Hunziker* considers that the dermal reactivity is predominant. In this, perhaps, lies the explanation of the fact that more than one epidermal application is often needed in order to obtain a positive test reaction.

Cutaneous parabiosis

A number of methods have been described for the passive transfer of an allergy of a sensitized animal to a normal animal. *Skog* (1955) has studied on a broader basis the passive transfer of allergic contact dermatitis. He succeeded in passively transferring a sensitivity to DNB with cells from peritoneal exudate, the thymus and the spleen as well as with lymph if a sufficient number of cells could be obtained. For this, 5—15 strongly sensitized donor animals were required. Similar results were obtained in parabiosis experiments, but this latter method seemed to be more reliable. For the purposes of the present study, therefore, parabiosis seemed to be the most suitable method. As no difference is considered to exist between cutaneous parabiosis and coelio-anastomosis the animals were connected by the skin and muscles alone.

In the present investigations sensitivity to chromium was passively transferred to a normal animal by cutaneous parabiosis. This succeeded in one experiment out of twelve. The results are meagre, but such experiments are attended by certain difficulties, including that of avoiding parabiosis intoxication. There is a great risk of this when the experiments are extended to a week or longer. In the present investigations the animals also presented lack of appetite and emaciation towards the end of the testing period. A tendency to partial separation could also be observed. In spite of such difficulties, one experiment out of twelve proved a definite success. It should be noted that the sensitization experiments with chromium and Freund's adjuvant did not lead to any noteworthy increased frequency of successful passive transfers of sensitivity to chromium.

CHAPTER V

GENERAL SUMMARY

(1) The object of the present work has been to study the effect of epidermal treatments of guinea pigs with potassium bichromate, to investigate whether the effect of potassium bichromate could be increased through the addition of a nonionic surface-active agent, La8Et (a lauryl alcohol-ethylene oxide condensate), and to investigate whether such epidermal treatments can lead to a sensitization to chromium.

(2) It emerged that potassium bichromate in a concentration of 0.0174 M has no immediate primarily irritant effect on guinea-pig skin, but that repeated treatments with this for at least seven days may cause local dermatites. After ten days 11 of 101 surviving animals had developed such skin changes.

(3) Similar treatment of guinea-pig skin with 0.0087 M La8Et caused no visible skin changes.

(4) A mixture of 0.0174 M potassium bichromate and 0.0087 M La8Et gave, in identical conditions with those in (2) above, dermatites as early as after five days' treatment and after ten days' treatment 50 of 100 surviving animals had developed such changes.

(5) The difference in the results which appeared in treatment with potassium bichromate and with potassium bichromate in combination with La8Et is, in the individual series of experiments, not always significant; this is due to the fact that the number of guinea pigs concerned in each experiment was relatively small. On aggregating the results from all series of experiments after seven and ten days' treatment, however, there appears a statistically established difference.

(6) The detailed effect that La8Et may have on the occurrence of these chromium dermatites is discussed.

(7) The dermatites which can be caused by these chromium solutions develop in such a way that they may seem to be the expression of a hypersensitivity to chromium but may also be supposed to be due to a chromium irritation. The latter is especially possible on the occasions when the dermatites become manifest after five days' treatment. Epidermal testing shows, however, that after the appearance of the dermatites the animals can present an increased skin reactivity to chromium. This is expressed in a shortened latency time for the appearance of the dermatites at the renewed exposure to chromium. An increased skin reactivity which is described, is considered to be the expression of a hypersensitivity to chromium. It does not differ from that which is obtained after intramuscular injections of potassium bichromate and complete Freund's adjuvant.

(8) An increased skin reactivity to potassium bichromate could, under favourable circumstances, passively be transferred in one of twelve experiments to a normal animal through cutaneous parabiosis. In these investigations parabiosis intoxication constituted one of the great difficulties.

TABLES

Table 1. Earlier experiments to sensitize guinea pigs with nickel.

Mode of sensitization	Nickel salt with/without detergent	Concentration	No. of animals with dermatites/total no. of animals	No. of animals with pos. test-reactions/total no. of animals			Authors
				Epicutaneous testing		Intracutan. testing	
				Single appl.	Repeated appl.		
Epidermal appl. daily for 14-20 days	NiSO ₄	1 %	31/31		6/6		Walthard 1926
Epidermal appl. daily for 7-28 days	NiCl ₂	2 %	dermatites in an unstated no. of 6 animals	0/6			Stewart & Cormia 1934
	NiCl ₂	10 %	dermatites in an unstated no. of 8 animals	0/8			
	NiCl ₂ Animals previously sensitized to horse serum	2 %	dermatites in the exp. animals	0/2			
Subcutaneous injection 0.1-0.2 ml every 3 days, 12 times in all	NiCl ₂	2 %			0/1	0/1	
Intradermal inj. 0.1 ml every 3 days, 12 times in all	NiCl ₂	2 %			0/2	0/3	
Intradermal inj. 0.4 ml x 1	NiCl ₂	2 %				0/5	
Intraperitoneal inj. 0.4 ml every 3 days, 7 times in all	NiCl ₂	2 %			uncertain reactions in 1/2		
Epidermal appl. in increasing conc. daily for 4-6 days	NiSO ₄	1-10 %	12/15	12/15			Graul & Kalkoff 1948
Epidermal appl. daily for 8 days	NiSO ₄	4 %	0/5	0/5			Nilzén & Wikström 1955
	NiSO ₄ + SDS	4 % 1 %	1/5	1/5			
Epidermal appl. daily for 10 days	NiSO ₄	0.5 %	0/6		0/6		Vinson 1959
	NiSO ₄	5.0 %	0/6		0/6		
	NiSO ₄ + SDS	0.5 % 1.0 %	pronounced dermatites in an unstated no. of animals		pronounced dermatites after 2 challenging doses. No. of animals not stated		
	NiSO ₄ + SDS	0.5 % 0.1 %	moderately pronounced dermatites in an unstated no. of animals		moderately pronounced dermatites after 2 challenging doses. No. of animals not stated		
	NiSO ₄ + DDBS	0.5 % 0.2 %	0/6		0/6		

Mode of sensitization	Nickel salt with/without detergent	Concen- tration	No. of animals with dermati- tes/total no. of animals	No. of animals with pos. test- reactions/total no. of animals			Authors
				Epicutaneous testing		Intra- cutan. testing	
				Single appl.	Repeated appl.		
Epidermal appl. daily for 10 days	NiSO ₄ + DDBS	0.5 % 1.0 %	pronounced dermatites in an unstated no. of animals		pronounced dermatites after 2 chal- lenging doses. No. of animals not stated		Vinson 1959
	NiSO ₄ + sodium acyl isethionate	0.5 % 1.0 %	0/6		0/6		
	NiSO ₄ + sodium acyl n-methyl taurate	0.5 % 1.0 %	0/6		0/6		
	NiSO ₄ + soap	0.5 % 8.0 %	slight irritation with moderate thickening		0/6		
	NiSO ₄ + tridecyl alcohol- ethylene oxide condensate	0.5 % 1.0 %	slight irritation		0/6		
	NiSO ₄ + lauryl alcohol- ethylene oxide condensate	0.5 % 1.0 %	0/6		0/6		
	NiSO ₄ + distearyl di- methyl ammo- nium chloride	0.5 % 1.0 %	0/6		0/6		
Epidermal appl. daily for 8 days	NiSO ₄ + SDS	4.0 % 1.0 %	not stated	0/3		0/3	Hunziker 1960
	NiSO ₄ + Triton X 100 Animals previous- ly sensitized to DNB	4.0 % 1.0 %	not stated	0/6 (1 uncer- tainly pos. reaction)		0/6	
	NiSO ₄ + Triton X 100	4.0 % 1.0 %	0/3			0/3	
Epidermal appl. twice daily for 3 days. Skin first irritated with savon noir	NiSO ₄ + Triton X 100	30.0 % 1.0 %	not stated	0/6		0/6	Hunziker 1960
Epidermal appl. 3 times daily until dermatites appear	NiSO ₄	1.0 %	dermatites	0/6		0/6	
Epidermal appl. 3 times daily until dermatites appear	NiSO ₄ previously DNB- sens. animals	1.0 %	dermatites	0/6		0/6	
Deep injection in neck twice with 1 week's interval	NiSO ₄ + Freund's adjuvant	4 mg NiSO ₄ in all ⁴		0/3		0/3	
Subcutaneous inj. x 1	suspension nickel Raney			0/6		0/6	

Table 2. Earlier experiments to sensitize guinea pigs with chromium.

Mode of sensitization	Chromium salt with/without detergent	Concentration	No. of animals with dermatites/total no. of animals	No. of animals with pos. test-reactions/total no. of animals			Authors
				Epicutaneous testing		Intra-cutan. testing	
				Single appl.	Repeated appl.		
Epidermal appl. daily for 8 days	K ₂ Cr ₂ O ₇	0.5 %	0/5	0/5			Nilzén & Wikström 1955
	K ₂ Cr ₂ O ₇ + SDS	0.5 % 1.0 %	5/5	5/5			
Epidermal appl. daily for 8 days	K ₂ Cr ₂ O ₇	0.5 %	0/2	0/2			Samitz & Pomerantz 1958
	K ₂ Cr ₂ O ₇ + SDS	0.5 % 1.0 %	4/4	0/4			
Epidermal appl. three times in all on alternate days	K ₂ Cr ₂ O ₇ + SDS	0.5 % 1.0 %	not stated	0/10			Mayer & Jaconia 1958
	CrCl ₃ , 6H ₂ O + SDS	0.5 % 1.0 %	not stated	0/10			
	Cr ₂ (OH) ₂ (SO ₄) ₂		not stated	1/10			
Intradermal inj. three times in all on alternate days	K ₂ Cr ₂ O ₇	0.025 %				5/10	
	CrCl ₃ , 6H ₂ O	0.025 %				10/10	
	Cr ₂ (OH) ₂ (SO ₄) ₂	0.025 %				10/10	
Epidermal appl. five times in all on alternate days	K ₂ Cr ₂ O ₇	1.0 %	not stated	0/12 number of challenging doses unknown			Levin, Brunner & Rattner 1959
	K ₂ Cr ₂ O ₇ after pretreatment with petrol. ether	1.0 %	not stated	6/12 number of challenging doses unknown			
Epidermal appl. daily for 8 days	K ₂ Cr ₂ O ₇ Triton X 100 or SDS	0.5 % 1.0 %	not stated	0/9 number of challenging doses unknown			Hunziker 1960
Deep injections twice	K ₂ Cr ₂ O ₇ Freund's adjuvant	1 mg K ₂ Cr ₂ O ₇ given in all		7/15 number of challenging doses unknown		15/15	

Table 3. Experiments to establish non-irritating concentrations of La 8 Et.

Concentration La 8 Et	No. of animals without or with reaction after											
	2 days				3 days				6 days			
	0	(+)	+	++	0	(+)	+	++	0	(+)	+	++
0.0347 M	5	0	0	0	4	1	0	0	3	1	1	0
0.347 M	0	0	5	0	0	0	1	4	0	0	0	5
0.694 M	0)	0	4	0	0	0	0	4	0	0	0	4

*) one animal died after 2 days

Table 4. Experiments to establish non-irritating concentrations of potassium bichromate.

Concentration $K_2Cr_2O_7$	No. of animals without or with reaction after															
	1 day				2 days				3 days				4 days			
	0	(+)	+	++	0	(+)	+	++	0	(+)	+	++	0	(+)	+	++
0.0174 M	18	0	0	0	18	0	0	0	18	0	0	0	18	0	0	0
0.0347 M	18	0	0	0	18	0	0	0	18	0	0	0	8	0	9	1
0.087 M	18	0	0	0	18	0	0	0	17	0	1	0	10)	0	7	1
0.174 M	18	0	0	0	16	0	2	0	8	0	7	3	1	0	5	12

Table 5. Treatment of guinea pigs with potassium bichromate + La 8 Et and with potassium bichromate alone.

Treatment with K ₂ Cr ₂ O ₇ + La 8 Et							Treatment with K ₂ Cr ₂ O ₇						
Macroscopic appearance after							Macroscopic appearance after						
Animal no.	5 days	6 days	7 days	8 days	9 days	10 days	Animal no.	5 days	6 days	7 days	8 days	9 days	10 days
Exp. 24/3 1958													
13	(+)	(+)	(+)	(+)	+	+	0	0	0	0	0	0	0
14	0	0	0	0	0	0	1	0	0	0	0	0	0
15	0	0	0	0	0	0	2	0	0	0	0	0	0
16	0	0	0	0	0	0	3	0	0	0	+	+	+
20	0	0	0	d. *)			4	0	0	0	0	0	0
21	0	0	0	0	0	0	5	0	0	0	0	0	0
22	0	0	0	(+)	+	++	6	0	0	0	0	0	0
23	0	0	0	0	0	0	10	0	0	0	0	0	0
24	(+)	(+)	(+)	(+)	(+)	++	11	0	0	0	0	0	0
25	0	0	0	0	0	(+)	12	0	0	0	0	0	0
Exp. 3/11 1958													
0	+	++	++	++	++	++	0	0	0	0	0	0	0
1	0	(+)	++	++	++	++	1	d. *)					
2	0	0	0	0	0	0	2	0	0	0	0	0	0
3	+	0	+	+	(+)	(+)	3	0	0	0	0	0	0
4	0	++	++	++	++	++	4	0	0	0	0	0	0
5	0	0	0	0	0	0	5	0	0	0	0	0	0
6	0	0	0	0	0	0	6	0	0	0	0	(+)	0
10	0	0	0	0	0	0	10	0	0	0	0	0	0
11	0	0	0	0	0	0	11	0	0	0	0	0	0
12	0	0	0	0	0	(+)	12	0	0	0	0	0	0
13	0	0	0	(+)	0	0	13	0	0	0	0	0	0
14	0	0	0	0	0	0							
Exp. 24/11 1958													
1	0	0	(+)	(+)	(+)	(+)	0	0	0	0	0	0	0
2	0	0	0	++	++	++	3	0	0	0	0	0	0
11	0	+	++	++	++	++	4	0	0	0	0	0	0
12	0	0	0	0	0	(+)	5	0	0	0	0	0	0
13	0	0	0	++	++	++	10	0	0	0	0	0	0
16	0	0	0	0	0	+							
Exp. 8/12 1958													
0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	1	0	0	0	0	0	0
2	0	0	0	0	(+)	(+)	2	0	0	0	0	0	0
3	0	0	0	(+)	++	++	3	0	0	0	0	0	0
4	0	0	0	0	(+)	0	4	0	0	0	0	0	0
5	0	0	0	0	0	0	5	0	0	0	0	0	0
6	0	0	0	(+)	0	++	6	0	0	0	0	0	0
10	0	0	0	0	+	(+)	10	0	0	0	0	0	0
11	0	0	0	0	+	(+)	11	0	0	0	0	0	0
12	0	0	0	0	+	(+)	12	0	0	0	0	0	0
0	0	0	0	0	+	+	5	0	0	0	0	0	0
1	0	0	0	0	++	++	6	0	0	0	0	0	0
2	0	0	0	0	0	d. *)	10	0	0	0	0	0	0
3	0	0	0	0	0	0	11	0	0	0	0	0	0
4	0	0	0	0	0	(+)	12	0	0	0	0	0	0
Exp. 10/1 1959													
15	0	0	+	++	++	++	0	0	(+)	(+)	+	++	++
22	0	0	0	0	(+)	(+)	1	0	0	0	0	+	+
23	0	0	+	++	++	++	2	0	0	0	0	(+)	(+)
24	0	0	0	0	0	0	3	0	0	0	+	++	++
25	+	++	++	++	++	++	4	0	(+)	(+)	+	++	++
26	0	0	(+)	+	++	++	5	0	0	0	0	(+)	(+)
30	0	0	+	++	++	++	6	0	0	0	0	0	0
31	0	(+)	+	++	++	++	10	0	0	0	0	0	0
32	0	+	(+)	++	++	++	11	0	0	0	0	0	0
33	0	0	(+)	0	0	0	12	0	0	0	(+)	0	0
34	0	0	(+)	++	++	++	13	0	0	0	0	0	0
35	0	0	0	0	0	0	14	0	0	0	0	(+)	(+)
40	(+)	++	++	++	++	++	16	0	0	0	0	0	0
41	0	0	0	0	0	0	20	0	0	0	++	++	++
							21	0	0	0	(+)	++	++

*) d = animal died

Treatment with $K_2Cr_2O_7$ + La 8 Et							Treatment with $K_2Cr_2O_7$						
Macroscopic appearance after							Macroscopic appearance after						
Animal no.	5 days	6 days	7 days	8 days	9 days	10 days	Animal no.	5 days	6 days	7 days	8 days	9 days	10 days
Exp. 26/1 1959													
15	0	(+)	0	(+)	(+)	+	0	0	0	0	0	0	(+)
22	+	++	++	++	++	++	1	0	0	0	0	0	0
23	0	0	0	0	0	0	2	0	0	0	0	0	0
24	0	0	0	+	+	++	3	0	0	0	0	0	0
25	0	(+)	0	++	++	++	4	0	0	0	0	0	(+)**)
26	0	0	0	0	0	0	5	0	0	0	0	0	0
30	0	0	0	(+)	+	+	6	0	0	0	0	0	(+)**)
31	0	(+)	0	0	0	0	10	0	0	(+)	(+)	+	++
32	0	0	0	0	0	(+)	11	0	0	0	0	0	(+)
33	0	0	0	(+)	+	+	12	0	0	(+)	+	+	++
34	0	0	0	0	0	0	13	0	0	0	0	0	0
35	+	(+)	+	+	+	++	14	0	0	+	+	++	++
36	0	(+)	+	++	++	++	16	0	0	0	0	0	0
40	0	(+)	+	+	++	++	20	0	0	0	0	0	(+)**)
41	0	0	0	0	+	+	21	0	0	0	0	0	0
Exp. 20/2 1959													
20	0	0	0	0	0	(+)	0	0	0	0	0	0	0
22	0	0	0	(+)	+	++	1	0	0	0	0	0	0
23	0	0	0	(+)	+	+	2	0	0	0	0	0	0
24	0	0	0	(+)	+	++	3	0	0	0	0	0	0
25	0	0	0	0	0	++	4	0	0	0	0	0	0
26	0	0	0	0	0	0	5	0	0	0	0	0	0
30	0	0	0	0	0	0	6	0	0	0	0	0	0
31	0	0	0	0	0	0	10	0	0	0	0	0	0
32	0	0	0	0	0	0	11	0	0	0	0	0	0
33	0	0	0	0	0	(+)	12	0	0	0	0	0	(+)
34	0	0	0	0	0	0	13	0	0	0	0	0	0
35	0	0	0	0	0	0	14	0	0	0	0	0	0
36	0	0	0	0	0	0	15	0	0	0	0	0	0
40	0	0	0	0	0	(+)	16	0	0	0	0	0	0
41	0	0	0	0	0	0	21	0	0	0	0	0	0
Exp. 28/9 1959													
10	0	0	+	+	+	++	0	0	0	0	0	0	0
11	0	0	0	(+)	(+)	+	1	0	0	0	0	0	0
12	0	0	(+)	+	+	+	2	0	0	0	(+)	(+)	+
13	0	0	(+)	+	++	++	3	0	d. *)				
14	0	(+)	(+)	+	(+)	+	4	0	0	0	0	0	0
15	0	d. *)					5	0	0	0	0	0	0
16	0	0	(+)	+	0	0	6	0	0	0	0	0	0
Exp. 23/11 1959													
0	(+)	(+)	+	+	++	+	0	0	0	0	0	0	0
1	+	++	++	++	++	++	1	0	0	0	0	(+)	0
2	(+)	+	+	+	++	++	2	0	0	0	0	(+)	0
3	+	+	+	+	++	+	3	0	0	0	0	0	0
4	+	+	+	++	++	++	4	0	0	0	0	0	0
5	+	+	+	++	++	++	5	0	0	0	(+)	(+)	0
6	+	+	++	++	++	++	6	0	0	0	0	0	0
10	(+)	(+)	+	++	++	++	10	0	0	0	0	0	0
11	+	+	++	++	++	++	11	0	0	0	0	0	0
							12	0	0	0	0	0	0

*) d = animal died

**) a number of scratch marks in the area treated

Table 6. Treatment of guinea pigs with potassium bichromate + La 8 Et and with La 8 Et alone.

Treatment with $K_2Cr_2O_7$ + La 8 Et							Treatment with La 8 Et						
Macroscopic appearance after							Macroscopic appearance after						
Animal no.	5 days	6 days	7 days	8 days	9 days	10 days	Animal no.	5 days	6 days	7 days	8 days	9 days	10 days
Exp. 24/3 1958													
13	(+)	(+)	(+)	(+)	+	+	26	0	0	0	0	0	0
14	0	0	0	0	0	0	30	0	0	0	0	0	0
15	0	0	0	0	0	0	31	0	0	0	0	0	0
16	0	0	0	0	0	0	32	0	0	0	0	0	0
20	0	0	0	d. *)			33	0	0	0	0	0	0
21	0	0	0	0	0	0	34	0	0	0	0	0	0
22	0	0	0	(+)	+	++	35	0	0	0	0	0	0
23	0	0	0	0	0	0	36	0	0	0	0	0	0
24	(+)	(+)	(+)	(+)	(+)	++	40	0	0	0	0	0	0
25	0	0	0	0	0	(+)	41	0	0	0	0	0	0
Exp. 3/11 1958													
0	+	++	++	++	++	++	0	0	0	0	0	0	0
1	0	(+)	++	++	++	++	1	0	0	0	0	0	0
2	0	0	0	0	0	0	2	0	0	0	0	0	0
3	+	0	+	+	(+)	(+)	3	0	0	0	0	0	0
4	0	++	++	++	++	++	4	0	0	0	0	0	0
5	0	0	0	0	0	0	5	0	0	0	0	0	0
6	0	0	0	0	0	0	6	0	0	0	0	0	0
10	0	0	0	0	0	0	10	0	0	0	0	0	0
11	0	0	0	0	0	0	11	0	0	0	0	0	0
12	0	0	0	0	0	(+)	12	0	0	0	0	0	0
13	0	0	0	(+)	0	0	13	0	0	0	0	0	0
14	0	0	0	0	0	0	14	0	0	0	0	0	0
Exp. 23/11 1959													
0	(+)	(+)	+	+	++	+	1	0	0	0	0	0	0
1	+	++	++	++	++	++	2	0	0	0	0	0	0
2	(+)	+	+	+	++	++	3	0	0	0	0	0	0
3	+	+	+	+	++	+	4	0	0	0	0	0	0
4	+	+	+	++	++	++	5	0	0	0	0	0	0
5	+	+	+	++	++	++	6	0	0	0	0	0	0
6	+	+	++	++	++	++	10	0	0	0	0	0	0
10	(+)	(+)	+	++	++	++	11	0	0	0	0	0	0
11	+	+	++	++	++	++	12	0	0	0	0	0	0

*) d = animal died

Table 7—15. Results of treatment with potassium bichromate + La 8 Et and with potassium bichromate alone.

	Treatment with $K_2Cr_2O_7$ + La 8 Et					Treatment with $K_2Cr_2O_7$				
	Macroscopic appearance				Animal which died	Macroscopic appearance				Animal which died
	0	(+)	+	++		0	(+)	+	++	
No. of animals after 5 days	8	2	0	0		10	0	0	0	
" " " " 6 "	8	2	0	0		10	0	0	0	
" " " " 7 "	8	2	0	0		10	0	0	0	
" " " " 8 "	6	3	0	0	1 died	9	0	1	0	
" " " " 9 "	6	1	2	0	1 "	9	0	1	0	
" " " " 10 "	5	1	1	2	1 "	9	0	1	0	

Table 8.
Exp. 3/11 1958

	Treatment with $K_2Cr_2O_7$ + La 8 Et					Treatment with $K_2Cr_2O_7$				
	Macroscopic appearance				Animal which died	Macroscopic appearance				Animal which died
	0	(+)	+	++		0	(+)	+	++	
No. of animals after 5 days	10	0	2	0		10	0	0	0	1 died
" " " " 6 "	9	1	0	2		10	0	0	0	1 "
" " " " 7 "	8	0	1	3		10	0	0	0	1 "
" " " " 8 "	7	1	1	3		10	0	0	0	1 "
" " " " 9 "	8	1	0	3		9	1	0	0	1 "
" " " " 10 "	7	2	0	3		10	0	0	0	1 "

Table 9.
Exp. 24/11 1958

	Treatment with $K_2Cr_2O_7$ + La 8 Et					Treatment with $K_2Cr_2O_7$				
	Macroscopic appearance				Animal which died	Macroscopic appearance				Animal which died
	0	(+)	+	++		0	(+)	+	++	
No. of animals after 5 days	6	0	0	0		5	0	0	0	
" " " " 6 "	5	0	1	0		5	0	0	0	
" " " " 7 "	4	1	0	1		5	0	0	0	
" " " " 8 "	2	1	0	3		5	0	0	0	
" " " " 9 "	2	1	0	3		5	0	0	0	
" " " " 10 "	0	3	1	2		5	0	0	0	

Table 10.
Exp. 8/12 1958

	Treatment with $K_2Cr_2O_7$ + La 8 Et					Treatment with $K_2Cr_2O_7$				
	Macroscopic appearance				Animal which died	Macroscopic appearance				Animal which died
	0	(+)	+	++		0	(+)	+	++	
No. of animals after 5 days	15	0	0	0		15	0	0	0	
" " " " 6 "	15	0	0	0		15	0	0	0	
" " " " 7 "	15	0	0	0		15	0	0	0	
" " " " 8 "	13	2	0	0		15	0	0	0	
" " " " 9 "	7	2	4	2		15	0	0	0	
" " " " 10 "	5	5	1	3	1 died	15	0	0	0	

Table 11.

Exp. 10/1 1959

	Treatment with $K_2Cr_2O_7$ + La 8 Et					Treatment with $K_2Cr_2O_7$				
	Macroscopic appearance				Animal which died	Macroscopic appearance				Animal which died
	0	(+)	+	++		0	(+)	+	++	
No. of animals after 5 days	12	1	1	0		15	0	0	0	
" " " " 6 "	10	1	1	2		13	2	0	0	
" " " " 7 "	4	4	4	2		13	2	0	0	
" " " " 8 "	5	0	1	8		9	2	3	1	
" " " " 9 "	4	1	0	9		6	3	1	5	
" " " " 10 "	4	1	0	9		6	3	1	5	

Table 12.

Exp. 26/1 1959

	Treatment with $K_2Cr_2O_7$ + La 8 Et					Treatment with $K_2Cr_2O_7$				
	Macroscopic appearance				Animal which died	Macroscopic appearance				Animal which died
	0	(+)	+	++		0	(+)	+	++	
No. of animals after 5 days	13	0	2	0		15	0	0	0	
" " " " 6 "	8	6	0	1		15	0	0	0	
" " " " 7 "	11	0	3	1		12	2	1	0	
" " " " 8 "	6	3	3	3		12	1	2	0	
" " " " 9 "	5	1	5	4		12	0	2	1	
" " " " 10 "	4	1	4	6		7	5	0	3	

Table 13.

Exp. 20/2 1959

	Treatment with $K_2Cr_2O_7$ + La 8 Et					Treatment with $K_2Cr_2O_7$				
	Macroscopic appearance				Animal which died	Macroscopic appearance				Animal which died
	0	(+)	+	++		0	(+)	+	++	
No. of animals after 5 days	15	0	0	0		15	0	0	0	
" " " " 6 "	15	0	0	0		15	0	0	0	
" " " " 7 "	15	0	0	0		15	0	0	0	
" " " " 8 "	12	3	0	0		15	0	0	0	
" " " " 9 "	12	0	3	0		15	0	0	0	
" " " " 10 "	8	3	1	3		14	1	0	0	

Table 14.
Exp. 28/9 1959

	Treatment with $K_2Cr_2O_7$ + La 8 Et					Treatment with $K_2Cr_2O_7$				
	Macroscopic appearance				Animal which died	Macroscopic appearance				Animal which died
	0	(+)	+	++		0	(+)	+	++	
No. of animals after 5 days	7	0	0	0		7	0	0	0	
" " " " 6 "	5	1	0	0	1 died	6	0	0	0	1 died
" " " " 7 "	1	4	1	0	1 "	6	0	0	0	1 "
" " " " 8 "	0	1	5	0	1 "	5	1	0	0	1 "
" " " " 9 "	1	2	2	1	1 "	5	1	0	0	1 "
" " " " 10 "	1	0	3	2	1 "	5	0	1	0	1 "

Table 15.
Exp. 23/11 1959

	Treatment with $K_2Cr_2O_7$ + La 8 Et					Treatment with $K_2Cr_2O_7$				
	Macroscopic appearance				Animal which died	Macroscopic appearance				Animal which died
	0	(+)	+	++		0	(+)	+	++	
No. of animals after 5 days	0*	3	6	0		10	0	0	0	
" " " " 6 "	0	2	6	1		10	0	0	0	
" " " " 7 "	0	0	6	3		10	0	0	0	
" " " " 8 "	0	0	3	6		9	1	0	0	
" " " " 9 "	0	0	0	9		7	3	0	0	
" " " " 10 "	0	0	2	7		10	0	0	0	

Table 16. Summary of all 9 experiments in which guinea pigs were treated with potassium bichromate + La 8 Et and with potassium bichromate alone.

	Treatment with $K_2Cr_2O_7$ + La 8 Et					Treatment with $K_2Cr_2O_7$				
	Macroscopic appearance				Animal which died	Macroscopic appearance				Animal which died
	0	(+)	+	++		0	(+)	+	++	
No. of animals after 5 days	86	6	11	0		102	0	0	0	1 died
" " " " 6 "	75	13	8	6	1 died	99	2	0	0	2 "
" " " " 7 "	66	11	15	10	1 "	96	4	1	0	2 "
" " " " 8 "	51	14	13	23	2 "	89	5	6	1	2 "
" " " " 9 "	45	9	16	31	2 "	83	8	4	6	2 "
" " " " 10 "	34	16	13	37	3 "	81	9	3	8	2 "

Table 17. Guinea pigs from 3 suppliers. Distribution of animals with and without positive dermatites after treatment with potassium bichromate.

	Strain I *)				Strain II **)				Strain III			
	Animals treated with:											
	K ₂ Cr ₂ O ₇ + La 8 Et		K ₂ Cr ₂ O ₇		K ₂ Cr ₂ O ₇ + La 8 Et		K ₂ Cr ₂ O ₇		K ₂ Cr ₂ O ₇ + La 8 Et		K ₂ Cr ₂ O ₇	
	pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.
No. of animals after 5 days	0	10	0	10	5	79	0	82	6	3	0	10
" " " " 6 "	0	10	0	10	7	76	0	81	7	2	0	10
" " " " 7 "	0	10	0	10	16	67	1	80	9	0	0	10
" " " " 8 "	0	9	1	9	27	56	6	75	9	0	0	10
" " " " 9 "	2	7	1	9	36	47	9	72	9	0	0	10
" " " " 10 "	3	6	1	9	38	44	10	71	9	0	0	10

*) one animal died

**) four animals died

Table 18. Results after 5, 7 and 10 days' treatment with potassium bichromate + La 8 Et and with potassium bichromate alone. Statistical analysis.

	No. of positive and negative animals after 5 days					
	Animal painted with $K_2Cr_2O_7 + La\ 8\ Et$		Animal painted with $K_2Cr_2O_7$		χ	Statistical significance
	pos.	neg.	pos.	neg.		
Exp. 24/3 1958	0	10	0	10	0	—
3/11 1958	2	10	0	10	1.35	—
24/11 1958	0	6	0	5	0	—
8/12 1958	0	15	0	15	0	—
10/1 1959	1	13	0	15	0.056	—
26/1 1959	2	13	0	15	1.464	—
20/2 1959	0	15	0	15	0	—
28/9 1959	0	7	0	7	0	—
23/11 1959	6	3	0	10	3.122	xx
Total	11	92	0	102	t=2.331	x
	No. of positive and negative animals after 7 days					
	Animal painted with $K_2Cr_2O_7 + La\ 8\ Et$		Animal painted with $K_2Cr_2O_7$		χ	Statistical significance
	pos.	neg.	pos.	neg.		
Exp. 24/3 1958	0	10	0	10	0	—
3/11 1958	4	8	0	10	2.018	x
24/11 1958	1	5	0	5	0.957	—
8/12 1958	0	15	0	15	0	—
10/1 1959	6	8	0	15	2.847	xx
26/1 1959	4	11	1	14	1.470	—
20/2 1959	0	15	0	15	0	—
28/9 1959	1	5	0	6	1.045	—
23/11 1959	9	0	0	10	4.359	xxx
Total	25	77	1	100	t=4.232	xxx
	No. of positive and negative animals after 10 days					
	Animal painted with $K_2Cr_2O_7 + La\ 8\ Et$		Animal painted with $K_2Cr_2O_7$		χ	Statistical significance
	pos.	neg.	pos.	neg.		
Exp. 24/3 1958	3	6	1	9	1.246	—
3/11 1958	3	9	0	10	1.701	—
24/11 1958	3	3	0	5	1.855	—
8/12 1958	4	10	0	15	2.230	x
10/1 1959	9	5	6	9	1.308	—
26/1 1959	10	5	3	12	2.589	xx
20/2 1959	4	11	0	15	2.148	x
28/9 1959	5	1	1	5	2.309	x
23/11 1959	9	0	0	10	4.359	xxx
Total	50	50	11	90	t=6.578	xxx

Table 19—21. Results of treatment with potassium bichromate + La 8 Et and with La 8 Et alone.
Exp. 24/3 1958

	Treatment with $K_2Cr_2O_7$ + La 8 Et					Treatment with La 8 Et				
	Macroscopic appearance				Animal which died	Macroscopic appearance				Animal which died
	0	(+)	+	++		0	(+)	+	++	
No. of animals after 5 days	8	2	0	0		10	0	0	0	
" " " " 6 "	8	2	0	0		10	0	0	0	
" " " " 7 "	8	2	0	0		10	0	0	0	
" " " " 8 "	6	3	0	0	1 died	10	0	0	0	
" " " " 9 "	6	1	2	0	1 "	10	0	0	0	
" " " " 10 "	5	1	1	2	1 "	10	0	0	0	

Table 20.
Exp. 3/11 1958

	Treatment with $K_2Cr_2O_7$ + La 8 Et					Treatment with La 8 Et				
	Macroscopic appearance				Animal which died	Macroscopic appearance				Animal which died
	0	(+)	+	++		0	(+)	+	++	
No. of animals after 5 days	10	0	2	0		12	0	0	0	
" " " " 6 "	9	1	0	2		12	0	0	0	
" " " " 7 "	8	0	1	3		12	0	0	0	
" " " " 8 "	7	1	1	3		12	0	0	0	
" " " " 9 "	8	1	0	3		12	0	0	0	
" " " " 10 "	7	2	0	3		12	0	0	0	

Table 21.
Exp. 23/11 1959

	Treatment with $K_2Cr_2O_7$ + La 8 Et					Treatment with La 8 Et				
	Macroscopic appearance				Animal which died	Macroscopic appearance				Animal which died
	0	(+)	+	++		0	(+)	+	++	
No. of animals after 5 days	0	3	6	0		9	0	0	0	
" " " " 6 "	0	2	6	1		9	0	0	0	
" " " " 7 "	0	0	6	3		9	0	0	0	
" " " " 8 "	0	0	3	6		9	0	0	0	
" " " " 9 "	0	0	0	9		9	0	0	0	
" " " " 10 "	0	0	2	7		9	0	0	0	

Table 22. Summary of 3 experiments in which guinea pigs were treated with potassium bichromate + La 8 Et and with La 8 Et alone.

	Treatment with $K_2Cr_2O_7$ + La 8 Et					Treatment with La 8 Et				
	Macroscopic appearance				Animal which died	Macroscopic appearance				Animal which died
	0	(+)	+	++		0	(+)	+	++	
No. of animals after 5 days	18	5	8	0		31	0	0	0	
" " " " 6 "	17	5	6	3		31	0	0	0	
" " " " 7 "	16	2	7	6		31	0	0	0	
" " " " 8 "	13	4	4	9	1 died	31	0	0	0	
" " " " 9 "	14	2	2	12	1 "	31	0	0	0	
" " " " 10 "	12	3	3	12	1 "	31	0	0	0	

Table 23. Results after 5, 7 and 10 days' treatment with potassium bichromate + La 8 Et and with La 8 Et alone. Statistical analysis.

	No. of animals with and without positive reactions after 5 days					
	Animal painted with $K_2Cr_2O_7$ + La 8 Et		Animal painted with La 8 Et		χ	Statistical significance
	pos.	neg.	pos.	neg.		
Exp. 24/3 1958	0	10	0	10	0	—
3/11 1958	2	10	0	12	1.477	—
23/11 1959	6	3	0	9	3.000	xxx
Total	8	23	0	31	3.215	xxx
No. of animals with and without positive reactions after 7 days						
Exp. 24/3 1958	0	10	0	10	0	—
3/11 1958	4	8	0	12	2.191	x
23/11 1959	9	0	0	9	4.243	xxx
Total	13	18	0	31	4.056	xxx
No. of animals with and without positive reactions after 10 days						
Exp. 24/3 1958	3	6	0	10	1.999	x
3/11 1958	3	9	0	12	1.633	—
23/11 1959	9	0	0	9	4.243	xxx
Total	15	15	0	31	4.534	xxx

Table 24. Testing with potassium bichromate. Guinea pigs were pretreated with epidermal applications of potassium bichromate.

Animal no.	Pretreatment with $K_2Cr_2O_7$						Testing with $K_2Cr_2O_7$ on the contralateral side 14 days after comm. of exp.		
	Macroscopic appearance after						Macroscopic appearance after		
	5 days	6 days	7 days	8 days	9 days	10 days	1 day	2 days	3 days
Exp. 10/1 -59									
0	0	(+)	(+)	+	++	++	0	(+)	+
1	0	0	0	0	+	+	0	(+)	+
2	0	0	0	0	(+)	(+)	0	0	0
3	0	0	0	+	++	++	0	(+)	+
4	0	(+)	(+)	+	++	++	0	(+)	+
5	0	0	0	0	(+)	(+)	0	0	0
6	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0
11	0	0	0	(+)	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0
14	0	0	0	0	(+)	(+)	0	0	0
16	0	0	0	0	0	0	0	0	0
20	0	0	0	++	++	++	0	(+)	+
21	0	0	0	(+)	++	++	0	0	+
Exp. 24/11-58									
0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0
Exp. 28/9 -59									
0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0
2	0	0	0	(+)	(+)	+	0	0	0
3	0	d							
4	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0

d = animal died

Table 25. Testing with potassium bichromate. Guinea pigs were pretreated with epidermal applications of potassium bichromate + La8Et.

Animal no.	Pretreatment with $K_2Cr_2O_7$ + La 8 Et						Testing with $K_2Cr_2O_7$ on the contralateral side 14 days after comm. of exp.		
	Macroscopic appearance after						Macroscopic appearance after		
	5 days	6 days	7 days	8 days	9 days	10 days	1 day	2 days	3 days
Exp. 24/11-58									
1	0	0	(+)	(+)	(+)	(+)	0	0	0
2	0	0	0	++	++	++	0	0	0
11	0	+	++	++	++	(+)	0	0	0
12	0	0	0	0	0	(+)	0	0	0
13	0	0	0	++	++	++	0	0	0
16	0	0	0	0	0	+	0	0	0
0	0	0	0	(+)	(+)	(+)	0	0	0
11	0	0	0	(+)	(+)	(+)	0	0	0
2	0	++	++	++	++	++	0	0	0
3	0	++	++	++	++	++	0	0	0
4	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
Exp. 10/1 -59									
15	0	0	+	++	++	++	0	0	(+)
22	0	0	0	0	(+)	(+)	0	0	0
23	0	0	+	++	++	++	0	(+)	+
24	0	0	0	0	0	0	0	0	0
25	+	++	++	++	++	++	0	(+)	+
26	0	0	(+)	+	++	++	0	(+)	+
30	0	0	+	++	++	++	0	(+)	+
31	0	(+)	+	++	++	++	0	(+)	+
32	0	+	(+)	++	++	++	0	0	(+)
33	0	0	(+)	0	0	0	0	0	0
34	0	0	(+)	++	++	++	0	(+)	+
35	0	0	0	0	0	0	0	0	0
40	(+)	++	++	++	++	++	0	(+)	+
41	0	0	0	0	0	0	0	0	0
Exp. 28/9 -59									
10	0	0	+	+	+	++	0	0	(+)
11	0	0	0	(+)	(+)	+	0	0	0
12	0	0	(+)	+	+	+	0	0	0
13	0	0	(+)	+	++	++	0	0	0
14	0	(+)	(+)	+	(+)	+	0	0	0
15	0	d					0	0	0
16	0	0	(+)	+	0	0	0	0	0
Exp. 23/11-59									
0	(+)	(+)	+	+	++	+	0	0	0
1	+	++	++	++	++	++	0	0	0
2	(+)	+	+	+	++	++	0	0	0
3	+	+	+	+	++	+	0	0	(+)
4	+	+	+	++	++	++	0	0	0
5	+	+	+	++	++	++	0	0	0
6	+	+	++	++	++	++	0	0	+
10	(+)	(+)	+	++	++	++	0	0	0
11	+	+	++	++	++	++	0	0	+
Exp. 22/11-60									
0	0	0	0	0	0	0	0	0	+
1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	(+)	0	0	0
3	0	0	+	+	+	+	0	+	++
4	0	0	0	+	+	(+)	0	0	0
5	0	0	+	+	+	++	0	(+)	+
6	0	0	(+)	(+)	(+)	(+)	0	0	0
10	0	0	+	+	+	++	0	0	0
Exp. 5/12-60									
30	(+)	+	(+)	+	+	+	0	+	+
31	0	(+)	(+)	+	+	(+)	0	0	0
32	0	0	0	(+)	(+)	(+)	0	+	+
33	0	0	0	(+)	+	+	0	0	0
34	0	(+)	0	+	+	(+)	0	0	0
35	0	+	+	++	++	++	0	(+)	+
36	0	0	0	0	0	0	0	0	0
40	(+)	+	+	+	+	+	0	0	(+)
41	0	0	0	0	0	0	0	0	0
42	(+)	+	+	++	++	++	0	+	++
43	0	(+)	(+)	+	+	+	0	+	+

Table 26. Testing with La8Et and with potassium bichromate + La8Et. Guinea pigs were pretreated with potassium bichromate + La8Et.

Animal	Pretreatment with $K_2Cr_2O_7$ + La 8 Et						Testing with La 8 Et			Testing with $K_2Cr_2O_7$ + La 8 Et		
	Macroscopic appearance after						Macroscopic appearance after			Macroscopic appearance after		
	5 days	6 days	7 days	8 days	9 days	10 days	1 day	2 days	3 days	1 day	2 days	3 days
Exp. 22/11-60							Exp. 10/1 -61			Exp. 10/1 -61		
0	0	0	0	0	0	0	0	0	0	0	0	(+)
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	(+)	0	0	0	0	0	0
3	0	0	+	+	+	+	0	0	0	0	0	(+)
4	0	0	0	+	+	(+)	0	0	0	0	0	(+)
5	0	0	+	+	+	++	0	0	0	0	0	+
6	0	0	(+)	(+)	(+)	(+)	0	0	0	0	0	0
10	0	0	+	+	+	++	0	0	0	0	0	(+)
Exp. 5/12-60							Exp. 11/1 -61			Exp. 11/1 -61		
30	(+)	+	(+)	+	+	+	0	0	0	0	+	+
31	0	(+)	(+)	+	+	(+)	0	0	0	0	0	0
32	0	0	0	(+)	(+)	(+)	0	0	0	0	0	(+)
33	0	0	0	(+)	+	+	0	0	0	0	(+)	+
34	0	(+)	0	+	+	(+)	0	0	0	0	(+)	(+)
35	0	+	+	++	++	++	0	0	0	0	+	+
36	0	0	0	0	0	0	0	0	0	0	0	0
40	(+)	+	+	+	+	+	0	0	0	0	+	+
41	0	0	0	0	0	0	0	0	0	0	0	0
42	(+)	+	+	++	++	++	0	0	0	0	++	++
43	0	(+)	(+)	+	+	+	0	0	0	0	++	++

Table 27. Testing with potassium bichromate. Guinea pigs were pretreated with intramuscular injections of potassium bichromate + Freund's adjuvant.

Animal no.	Testing with $K_2Cr_2O_7$		
	Macroscopic appearance after		
	1 day	2 days	3 days
Exp. 6/7 -61			
20	0	+	+
21	(+)	+	+
22	(+)	(+)	(+)
23	0	0	0
24	+	+	+
25	(+)	+	+
26	0	+	+
30	d		
31	0	(+)	+
32	(+)	+	+
34	0	(+)	(+)
35	+	+	+
Exp. 7/7 -61			
0	0	0	0
1	(+)	0	(+)
15	0	(+)	(+)
Exp. 29/5 -61			
0	0	0	0
1	0	0	0
2	0	(+)	+
3	0	(+)	+
4	0	0	+
Exp. 26/9 -61			
0	0	0	0
5	0	(+)	+
6	0	0	(+)
11	0	0	0
12	0	0	(+)
14	0	0	0
16	0	0	0
20	0	+	+
Animal no.	Testing with $K_2Cr_2O_7$		
	Macroscopic appearance after		
	1 day	2 days	3 days
Exp. 5/10-61			
4	0	0	0
5	0	(+)	(+)
6	0	0	(+)
10	0	0	0
Exp. 6/10-61			
21	0	+	+
22	0	+	+
23	d		
24	0	0	0
25	0	+	(+)
26	0	+	+
30	0	0	(+)
Exp. 30/10-61			
0	0	0	(+)
2	0	0	(+)
3	0	+	+
4	(+)	(+)	+
5	+	+	++
6	0	0	(+)
10	(+)	(+)	+
11	0	0	(+)
20	(+)	+	+
21	0	(+)	(+)
Exp. 14/8 -61			
0	0	+	+
1	0	(+)	(+)
2	0	0	(+)
3	0	0	(+)
4	0	0	(+)
5	0	0	+
6	0	0	0
Exp. 22/8 -61			
54	0	(+)	+
55	0	(+)	+
56	0	(+)	+

d = animal died

Table 28. Parabiosis experiments in which the donor had been pretreated with epidermal applications of potassium bichromate + La8Et.

Treatment with $K_2Cr_2O_7$ + La 8 Et							
Donor				Recipient			
Date	Macroscopic appearance after			Animal no.	Macroscopic appearance after		
Animal no.	1 day	2 days	3 days		1 day	2 days	3 days
Op. 23/10-59							
10	0	0	0	0	0	0	0
Op. 27/1 -60							
5	0	0	0	10	0	0	0
Op. 3/3 -60							
4	0	0	+	6	0	0	+
Op. 2/6 -60							
11	0	0	0	5	0	0	0
Op. 16/6 -60							
13	0	0	0	3	0	0	0
Op. 4/7 -60							
14	0	0	(+)	0	0	0	0
Op. 5/7 -60							
15	0	0	0	10	0	0	0
Op. 17/5 -60							
0	0	0	0	4	0	0	0
Op. 18/7 -60							
6	0	0	0	3	0	0	0
Op. 16/8 -60							
10	0	0	+	12	0	0	0
Op. 25/8 -60							
20	0	0	0	0	0	0	0
Op. 23/9 -60							
22	0	0	0	0	0	0	0

Table 29. Parabiosis experiments in which the donor had been pretreated with potassium bichromate + Freund's adjuvant.

Treatment with $K_2Cr_2O_7$ + La 8 Et							
Donor				Recipient			
Date	Macroscopic appearance after			Animal no.	Macroscopic appearance after		
Animal no.	1 day	2 days	3 days		1 day	2 days	3 days
Op. 31/5 -61	(this guinea pig became increasingly emaciated and was destroyed without further testing)			0	(no testing as the donor had been separated and destroyed)		
2							
Op. 5/6 -61							
3	+	+	+	0	0	0	0
Op. 6/6 -61							
4	0	0	d	0	0	0	(separation)
Op. 11/7 -61							
25		(not tested)		40	0	d	
Op. 17/7 -61							
31	+	+	+	0	+	+	+
Op. 12/9 -61							
13	0	0	0	61	0	0	0
Op. 13/9 -61							
15	0	0	(+)	60	0	(+)	(+)
Op. 31/10-61							
26	0	(+)	+	3	0	0	0
Op. 9/11-61							
6	0	(+)	(+)	2	0	+	+
Op. 22/11-61							
5	0	0	+	22	0	0	(+)
Op. 22/11-61							
20	0	(+)	(+)	14	0	(+)	(+)
Op. 23/11-61							
3	0	(+)	(+)	13	(+)	+	+
Op. 27/11-61							
11	0	0	0	23	0	0	(+)
Op. 28/11-61							
10	0	0	(+)	1	(+)	(+)	+
Op. 29/11-61							
4	0	0	0	24	(+)	(+)	(+)

d = animal died

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